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Evaluation of factors affecting avian risk from granular pesticides

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Evaluation of factors affecting avian risk from granular pesticides

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DEDICATION

To Brad

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION

Introduction

Many pesticides are formulated on granules made of various materials, such as sand, clay, gypsum, corncob, etc., and are used extensively in the United States to control pests in agricultural crops and on turf sites. Granular pesticides were developed in the 1960s to reduce the risk of exposure to workers during pesticide applications (U.S. EPA, 1992). Although the risk of pesticide exposure to humans may be reduced through the use of granular formulations, granular pesticides can be highly toxic to birds (Balcomb et al., 1984; Hill and Camardese, 1984), and avian mortalities following field applications of granular pesticides have been documented (U.S. EPA, 1989, 1992; Mineau, 1993).

In response to the concern over the potential avian risk from granular formulations, the U. S. Environmental Protection Agency (EPA) developed an index for acute avian risk to identify granular pesticides that might pose acute lethal risks to birds. The risk index, expressed as LD50/ft^2, is based on both the acute toxicity of a pesticide (the lethal dose of the pesticide that will kill 50% of a test population, or LD50) and the number of granules exposed on the ground after pesticide application. Based on this index, avian risk from granular pesticides was initially considered unacceptable if pesticide application resulted in more than 1.0 LD50/ft^2. EPA selected 1 LD50/ft^2 as the threshold level because field applications of 1 LD50/ft^2 were shown to have resulted in avian mortality (U.S. EPA, 1992). The threshold level has subsequently been decreased to 0.5 LD50/ft^2 (U.S. EPA, 1994).

Objections have been raised (unpubl. correspondence from the American Crop Protection Association (ACPA) to the EPA, Nov. 30, 1992) regarding the EPA's risk index because the index is based on several assumptions (U.S. EPA, 1992) regarding pesticide toxicity and potential exposure that have not been validated by scientific data. For example, the index assumes that adverse effects to birds will increase as granule availability increases per unit area. In addition, it is assumed that granule ingestion is accidental or random rather than intentional and that birds can consume enough granules to equal an LD50. Further, LD50/ft^2 calculations only use the pesticide-treated band as the unit area, rather than both the band and the area between bands, because it is assumed that the disturbed area is the focus of bird activity, and this can substantially increase the value of the risk index for a given granular pesticide. Because the risk index is based on assumptions that are unsubstantiated, there is a potential for inaccurate risk assessments.

Other objections to the risk index have been raised because factors besides the acute toxicity of a pesticide and the number of granules exposed per square foot may contribute to avian risk from granular pesticides, and these are not taken into account by the index. For example, all granular carrier types are currently evaluated as if they are the same, when they actually differ in shape, composition and surface texture. Such variations can affect whether or not a bird will intentionally ingest a pesticide granule (Best and Gionfriddo, 1994a,b). Granule ingestion by birds also can be affected by granule size and color (Gionfriddo and Best, 1995 and 1996). Further, other factors such as pesticide load per granule, soil color and texture, weather conditions, the number of pesticide applications per season, application methods, bird foraging behaviors, etc. are not considered when assessing risk using the current risk index (see Best and Fischer, 1992, for a discussion). Thus, it is improbable that the index accurately predicts the risk that granular pesticides pose to birds.

The risk index also is limited when estimating the number of granules exposed in a given unit area. The number of granules exposed on the soil surface after application can vary, depending on granule size and weight, configuration and calibration of equipment, field conditions, etc. Because the amount of granules exposed after application depends on several factors, which are mostly unaccounted for when calculating the number of exposed granules, the risk index "does not provide a definitive value for the amount of pesticide that will be available to birds" (U.S. EPA, 1992, p.18).

The purpose of this dissertation is to evaluate some of the limitations of the LD50/ft^2 risk index. Chapters 2 and 3 provide information regarding how factors other than acute toxicity and the number of granules exposed can contribute to avian risk. This is done by evaluating the effects of granule carrier type, pesticide load per granule, granule color, granule size, and soil moisture on avian risk. Chapter 4 investigates the relationship between avian risk and application rate and addresses the EPA's assumptions that avian risk increases in a linear fashion as granule availability increases. Chapter 5 is a comprehensive review of the experimental evidence regarding the influence of pesticide granule characteristics (composition, shape, surface texture, size, color, and pesticide load per granule) on avian risk and provides suggestions regarding 1) risk reduction through the development of granular pesticide formulations that are less hazardous to birds, 2) future research needs of bird response to granule characteristics, and 3) factors (e.g., pesticide granule characteristics) that could be included in a risk exposure model and how they might be represented in such a model.

Dissertation Organization

This dissertation consists of six chapters including a General Introduction (Chapter 1) and a General Conclusion (Chapter 6). Chapters 2 through 5 were prepared separately for submission to scientific journals for publication and were formatted according to journal style. Louis B. Best is a junior author on each paper and David L. Fischer is a junior author on one paper (Chapter 2).

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CHAPTER 2: EFFECTS OF DIFFERENT FORMULATIONS OF GRANULAR PESTICIDES ON BIRDS

A paper published in Environmental Toxicology and Chemistry*

Tamara R. Stafford, Louis B. Best, and David L. Fischer

Abstract–Fensulfothion was formulated on granular pesticide carriers to evaluate the effects of carrier type (silica, clay, and corncob), pesticide load per granule (1/2, 1/8, and 1/32 of the LD50), granule size (0.2 to 0.6 and 1.0 to 1.4 mm), and granule color (white and blue) on avian risk from granular pesticides. Carrier type and pesticide load per granule were evaluated in a separate experiment from granule size and color. In both experiments, captive house sparrows (*Passer domesticus*) were offered the granular pesticide formulations in bands on soil surfaces. Bird behavior, body weight, survival, and brain and plasma cholinesterase activity were used to assess pesticide exposure. Silica and clay carrier types posed a greater risk to birds than corncob granules. Exposure also was greater with a higher pesticide load per granule. Findings were inconclusive regarding the effect of granule size and color on avian exposure to granular pesticides. Precipitation seemed to increase the possibility of dermal exposure to the pesticide. It is important to consider factors in addition to those used currently by the EPA when evaluating the risk to birds from granular pesticides.

INTRODUCTION

Many pesticides are applied as granular formulations because they are safer and easier to apply and can be more effective against certain pests. Granular pesticides, however, can be highly toxic to birds [1,2], which puts many avian species at risk [3]. The Environmental

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Protection Agency (EPA) currently evaluates risk posed by granular pesticides on the basis of the pesticide's acute toxicity to birds and the estimated number of granules exposed after application. Using these two criteria, the EPA has developed a risk index expressed as "LD50/ft^2." Based on this index, avian risk from granular pesticides is considered potentially unacceptable if pesticide applications result in more than 0.5 LD50/ft^2 [4].

The EPA's risk index, however, does not take into account other factors that may contribute to avian risk from granular pesticides. For example, the current policy evaluates all granular carrier types as if they are the same when, in fact, they differ in composition, shape, and surface texture, and these variations can affect whether or not a bird will ingest a granule [5,6]. Granule ingestion also can be affected by granule size and color [7,8]. Other factors that may affect avian risk from pesticide granules include pesticide load per granule, soil color and texture, weather conditions, number of pesticide applications per season, application method, and rate of chemical uptake from different granular carrier types once ingested. Foraging behavior and habitat use also can determine if and how a bird may come in contact with granular pesticides. Potential routes of avian exposure to granular pesticides include granule ingestion, dermal absorption, and ingestion of prey or plant material containing pesticide residues [9]. The relative importance of these routes of exposure is often confounded because more than one route may be operating simultaneously.

It is important to evaluate each factor that may contribute to avian risk from granular pesticides. Given the number of factors, however, it is not possible to evaluate them all at once; consequently, we selected four variables that are potentially related to granule ingestion as the primary route of exposure. The purpose of our study was to document the effects of granular carrier type, pesticide load per granule, granule size, and granule color on avian risk. It was further hoped that the findings from our research would provide input to refine the risk index currently used.

METHODS

Study variables

Three commonly used granular pesticide carriers were selected for study: silica, a heat-treated montmorillonite clay, and corncob. Silica granules are quartz, a material consumed by free-ranging birds as grit [10,11]; consequently, birds may ingest silica granules as a source of grit. Corncob granules resemble corn fragments [12], a food source used by many birds [13], and these granules may be mistaken for food. Of the three granular carriers tested, clay granules most resemble soil particles and may be less visible on the soil surface. Birds may ingest these granules inadvertently while foraging. Each carrier type was sieved to obtain granules 0.6 to 1.0 mm in diameter, the size of grit most frequently consumed by free-ranging house sparrows (*Passer domesticus*) [14].

The pesticide load per granule was evaluated because this determines the number of granules a bird would have to consume to be adversely affected. Fensulfothion (*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate), an organophosphate pesticide, was selected for this study because it is highly toxic to birds [1,15] and was available for experimental formulations. Three different loads of fensulfothion were formulated on each carrier type, which represented 1/2, 1/8, and 1/32 of the LD50 (0.3 mg/kg of technical grade fensulfothion [1]) per granule for a 27-g house sparrow. Hereafter, the loads will be referred to as high, medium, and low, respectively. The granular pesticides were formulated by Bayer Corporation (formerly Miles Inc., Kansas City, MO, USA). An analysis of the percentage active ingredient conducted by Bayer Corporation for each formulation verified that the actual loads fell within 70 to 130% of the nominal, which the EPA considers an acceptable analytical recovery [4].

To test for size effects, silica granules of two sizes, small (0.2 to 0.6 mm) and large (1.0 to 1.4 mm), were used. The two sizes represented the upper and lower size range of grit normally consumed by house sparrows [14].

Color effects were evaluated with artificially-colored white and blue (FD&C Blue #1 Lake, Warner-Jenkinson, St. Louis, MO, USA) silica granules. Originally, color effects were to be evaluated by using uncolored and blue granules, however, after the "uncolored" granules were formulated with the pesticide, they became white due to the formulation process. Control granules were uncolored. White grit particles have been shown to be preferentially ingested by house sparrows whereas blue grit is avoided [7].

Test species

The house sparrow was chosen as the experimental species for several reasons. First, house sparrows are ground-foraging granivores [16], a feeding guild susceptible to granular pesticides. Second, they have a comparatively high grit consumption rate [8,14], which would increase the likelihood of granule consumption. Third, the use of house sparrows allowed test results to be compared with those from previous studies [5,6,7,8,12,14].

Test birds were captured in rural areas of Story County, IA, USA by using mist nets. Captured birds were weighed, marked with a numbered leg band, and held in an outdoor holding facility. Birds were acclimated to captivity for at least 7 d before being used in the study. A wild bird seed mix (Cardinal Brand Wild Bird Feed, Des Moines Feed Co., Des Moines, IA, USA) containing millet, milo, cracked corn, sunflower seeds, peanuts, and wheat was available to the birds *ad libitum*, and the water (Ames municipal water) was supplemented with a vitamin and electrolyte mix, prepared according to the manufacturer's directions (Biotin Stress Pak, Salsbury Laboratories, Inc., Charles City, IA, USA). The holding facility used for acclimation was roofed (wood) and enclosed with hardware cloth. The facility contained at least two feeding stations and two water stations and was equipped with perches and sheltered areas.

Study design

The effects of granular carrier type and pesticide load per granule were evaluated first by using a 3 (carrier: silica, clay, corncob) x 4 (load: high, medium, low, control) factorial design.

Unformulated granules (blanks) were used as controls. Treatments were randomly assigned to the 12 study aviaries (see below), and 15 house sparrows were randomly assigned to each treatment. The experiment was replicated three times, and each replicate ran for 3 d.

Based on preliminary evaluations, an application rate of 50 LD50/ft^2 was selected to evaluate carrier type and load per granule. Lower application rates of 1, 5, and 10 LD50/ft^2 did not cause adverse effects in preliminary tests. At 50 LD50/ft^2 there were sufficient measurable effects to show treatment-related differences in behavioral symptoms, mortality, and cholinesterase inhibition. For the carrier type by pesticide load experiment, the number of granules/ft^2 varied but the LD50/ft^2 was constant.

Granule size and color were subsequently evaluated by using a 2 (size: large, small) x 3 (color: blue, white, uncolored) factorial design. The number of birds per treatment, number of replicates, and experiment duration were the same as for the carrier type by pesticide load experiment. The high pesticide load formulated on silica granules was selected to test size and color effects because this formulation showed the greatest treatment-related effects (see below). The application rate was increased to 100 LD50/ft^2 because an application rate of 50 LD50/ft^2 in the carrier type by pesticide load experiment did not show as great an effect as anticipated. For the granule size by color experiment, both the number of granules/ft^2 and the LD50/ft^2 were constant across all treatments.

Both experiments were conducted in roofed (corrugated steel) outdoor aviaries that measured either 3.7 x 3.7 x 1.8 m or 3.7 x 4.6 x 2.1 m. Aviaries were enclosed with a combination of hardware cloth and fiberglass. Each aviary had a simulated soil surface at least 1.5 cm deep. Top soil was obtained from a site nearby and was screened to remove rocks and debris before being placed in the aviaries. Each aviary was supplied with one water station, and *ad libitum* feed was scattered evenly on the soil surface. About 1 kg of feed was scattered in each aviary 2 d before exposure (day -2), and depending on the amount left on application day (day 0), more feed was scattered immediately after pesticide application. The amount of feed added after application ranged from 0.0 to 0.5 kg. The granular pesticide was applied in bands 7 in (18 cm) wide and 36 in (90 cm) apart, spacing similar to normal row-crop applications. The pesticide bands were 3.6 m long in all aviaries, and each aviary had four bands, thus, the pesticide application area was consistent across all treatments, regardless of aviary size.

Before pesticide application, each band was marked by running a rope along each side of the band. The preweighed amount of pesticide was evenly distributed by hand to the band. To ensure even granule distribution, the pesticide for a particular band was divided into three portions and applied to 4-ft (120-cm) sections of the band. The ropes were removed immediately after pesticide application.

After application, exposed granules were counted in one randomly selected square foot of one band within each aviary, and a count was made each day thereafter at the same location. Counts of exposed granules were made for the silica treatments only. Counts were attempted for the clay and corncob treatments, but to the human eye the clay granules were indistinguishable from the soil and the corncob granules blended in with the scattered feed.

Maximum temperature and total precipitation were measured daily at a weather station 12 km west of the aviaries [17]. Sometimes when it rained, portions of the soil in some of the aviaries became wet. Although the aviary roofs protected the soil from direct rainfall, water tended to enter at the bottom edges of the aviaries during heavy rains. The maximum proportion of soil that became wet was recorded for each aviary for each experiment.

Pesticide exposure was assessed by recording bird body weight, behavior, mortality, and plasma and brain cholinesterase (ChE) activity. All test birds were weighed on day -2 and again 3 d after exposure (day 3), or when they were found dead, by using a 50-g Pesola scale. After pesticide application, birds were observed twice daily (midmorning and late afternoon) for abnormal behaviors that might indicate pesticide exposure. Birds were observed for at least 1 min per aviary, and more time was spent when needed (i.e., many of the birds in an aviary were exhibiting symptoms). The behaviors included: ataxia (muscular incoordination, i.e., inability to fly and impaired walking), asthenia (weakness, debility, easily fatigued), hyporeactivity (diminished reaction to stimuli, i.e., bird response to observer's movement was slower than normal), hyperexcitability (increased reaction to stimuli), pilorection (feathers held erect), catatonia (extreme unresponsiveness and inactivity, i.e., immobility), unkemptness (lack of preening), and wing drop (wing position abnormally low) [18]. Of the symptoms observed, only ataxia, hyporeactivity, and pilorection occurred with any regularity and, therefore, were the only ones that could be analyzed statistically. Any deaths occurring during the experiments were recorded, and the carcasses were collected for brain ChE analysis.

Blood samples were collected from live birds on days -2, 1, and 3 from eight birds per aviary. The eight birds were selected randomly on day -2, and blood was collected from the same birds on all three days, unless a bird died during the experiment. Samples (about 40 μL) were collected in a heparinized capillary tube from the ulnaris (wing) vein and kept on wet ice until centrifugation. Blood samples were centrifuged at 7500 RPMs for 20 min, and the plasma was stored at -70°C until analysis. All birds surviving to the end of the experiments were euthanized for brain ChE analysis.

ChE analysis

Plasma and brain ChE analyses were used to assess ChE inhibition resulting from exposure to the pesticide. Plasma was analyzed at Iowa State University according to methods of Ellman et al. [19], modified for use on a Vmax 96-well plate reader (Molecular Devices Corporation, Palo Alto, CA, USA). Reagent volumes used in the assay were 10 μL 0.042 M acetylthiocholine iodide (ATCI) as substrate and 20 μL 0.004 M 5,5-dithio-bis-(2-nitrobenzoic acid) in 250 μL of 0.1M Tris buffer (pH 8.0). Reagents and buffers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The assay sample volume was 5 μL plasma. Assays were conducted at 25°C and a wavelength of 405 nm; 15 absorbance readings were taken during a 2-min period. Three subsamples of each plasma sample were analyzed, and additional assays were conducted if the three subsamples differed by more than 15%. Plasma ChE activity was expressed in International Units (U/L) and represented the amount of ChE catalyzing the transformation of 1 μmol of substrate/min/L.

Brain samples were frozen and sent to the EPA Environmental Research Laboratory in Corvallis, OR, USA, for analysis. Whole brains were excised and homogenized in 0.1M Trizma buffer (pH 7.4) at the ratio of 35 mg brain/ml buffer by using a Virtis® "45" homogenizer (Virtis Co., Gardiner, NY, USA) at 30% power for 15 s. The entire homogenate for each brain was centrifuged for 20 min at 12,500 *g* by using a Sorval® RC-5 refrigerated (0 to 5°C) centrifuge. The supernatant was refrigerated (0 to 6°C) until assayed (within 5 h).

Methods for analyzing brain ChE activities were similar to plasma analysis methods. Reagent molarities and volumes were the same, except for the pH 8.0 Trizma buffer, where 200 μL were used rather than 250 μL. Sample volume was 10 μL supernatant. Assays were conducted at 37°C and at a wavelength of 405 nm. Twenty absorbance readings were taken during a 3-min period. Two subsamples were analyzed for each brain and additional assays were conducted if the coefficient of variation for the two subsamples was more than 15%. Brain ChE activity was expressed as μmols of substrate (ATCI) hydrolyzed/min/g tissue.

Data analysis

Because no significant sex differences were found for any of the dependent variables, the data from males and females were combined for subsequent analyses. Body weights on day 3 were analyzed by using two-way analysis of covariance (ANCOVA), with day -2 body weights as the covariate. In the carrier type by pesticide load experiment, a 3 (silica, clay, corncob) X 4 (high, medium, low, control) ANCOVA was used. In the size by color experiment, a 2 (large, small) X 3 (blue, white, uncolored) ANCOVA was used. Plasma ChE data were transformed to percent of control for statistical analysis. Because plasma ChE activities were measured on three separate days (i.e., day -2, day 1, day 3), these data were analyzed by using multivariate analysis of variance (MANOVA). MANOVA was used rather than repeated measures ANOVA because the statistical assumptions are less stringent for MANOVA than for the repeated measures ANOVA [20]. Brain ChE activity, mortality, and behavioral symptoms were analyzed by using two-way analysis of variance (ANOVA).

An alpha level of 0.05 was used for all statistical tests. When significant differences were found for independent variables with more than two categories (i.e., color, carrier type, load), Fisher's Least Significant Difference (LSD) test was used for follow-up post hoc analyses. Simple effects analyses were used to interpret significant interactions.

RESULTS

Carrier type by pesticide load experiment

In the carrier type by pesticide load experiment, the silica granules were rapidly incorporated into the soil after day 0 (Fig. 1a). Rapid incorporation resulted mainly from bird foraging activities, and although silica granule incorporation may have differed from that of clay and corncob granules, we suspect that the latter also were rapidly incorporated. Despite the rapid incorporation, there was at least 1 LD50/ft^2 in all the silica treatments throughout the experiment.

Pesticide load per granule significantly influenced bird behavior ($F[3,24]=12.25$, $p<0.01$). Significantly more birds in the high-load treatment (mean=0.78, S.E.=0.67) became ataxic, whereas no birds became ataxic in the controls, low-load, or medium-load treatments. There were no significant differences among loads regarding hyporeactivity or pilorection. Carrier type did not have a significant effect on bird behavior.

Carrier type influenced plasma ChE activities. There was a significant interaction between day of measurement and carrier type ($F[4,34]=3.69$, $p=0.02$). The effect of carrier type on plasma ChE was nonsignificant on day -2 ($F[2,18]=0.31$, $p=0.73$) but significant on days 1 and 3 ($F[2,18]=18.76$, $p<0.01$; and $F[2,18]=7.65$, $p<0.01$, respectively; Fig. 2). On day 1, plasma ChE activity of birds was significantly lower in aviaries with silica and clay carriers than in aviaries with corncob carriers. On day 3, plasma ChE activity was significantly lower in aviaries with clay carriers than in aviaries with silica and corncob carriers. Neither carrier type nor pesticide load significantly influenced brain ChE activity ($F[2,24]=1.55$, $p=0.23$; $F[3,24]=0.49$, $p=0.69$, respectively).

Mortality occurred in all treatments, including the controls, but neither carrier type ($F[2,16]=0.91$, $p=0.42$) nor pesticide load per granule ($F[3,16]=2.15$, $p=0.15$) seemed to influence mortality. The occurrence of mortality in the controls suggests that there were deaths related to causes other than treatment effects, which may have confounded treatment-related effects (Table 1). Although there were no significant differences in mortality among treatments, the likelihood that some birds died as a result of pesticide exposure is indicated by the brain ChE activities of birds found dead. Two birds found dead, one in the clay medium-load treatment and the other in the clay high-load treatment, had >50% brain ChE inhibition compared to control birds, which is strong evidence that the deaths of those individuals were due to anticholinesterase poisoning [21]. Neither carrier type ($F[2,23]=0.14$, $p=0.87$) nor pesticide load per granule ($F[3,23]=0.80$, $p=0.51$) significantly affected body weights.

Granule size by color experiment

As with the carrier type by pesticide load experiment, the granules in the size by color experiment were rapidly incorporated into the soil (Fig. 1b). Because the application rate for the size by color experiment had been increased from 50 LD50/ft^2 to 100/ft^2, there were more granules on the soil surface (at least four LD50/ft^2) available through day 3 in all treatments. The counts for the small granules were considerably less than those for the large granules, probably because the former were less detectable than the latter. Small granules also may have been more readily incorporated into the soil than the large granules.

In the size by color experiment, four birds (mean=0.44, S.E.=0.73) in the large granule treatments became ataxic, whereas no birds in the small granule treatments or in the controls became ataxic. This difference, which was nearly significant ($F[1,12]=4.00$, $p=0.07$), suggests that granule size may have influenced exposure. There was no behavioral evidence suggesting that granule color influenced exposure. As with the carrier type by pesticide load experiment, there were no significant differences between treatments for hyporeactivity or pilorection.

Although the behavioral data suggested that birds experienced greater adverse effects in the large granule treatments, the mortality data suggested that birds showed greater adverse effects ($F[1,12]=4.76$, $p=0.05$; Table 1) in the small granule treatments. There was no evidence that granule color influenced mortality ($F[2,12]=0.18$, $p=0.84$).

Statistical analysis of the plasma ChE data found no significant effects for size or color ($p's>0.23$). There was no evidence that size or color influenced body weight ($p's>0.21$) or brain ChE activity ($p's>0.66$).

DISCUSSION

The results of the carrier type by pesticide load experiment demonstrate that avian exposure to granular pesticides may be influenced by the type of carrier. In our experiment, silica and clay granules seemed to pose a greater risk of adverse effects to house sparrows than corncob granules. That the silica granules posed no greater risk than the clay granules was unexpected because previous work [5] indicated that house sparrows had a stronger preference for silica granules than for clay granules. It was not surprising that corncob granules posed less risk compared with silica and clay granules because corncob was one of the least preferred carrier types in the previous study.

It is not clear why corncob granules should present less risk of pesticide exposure compared with silica and clay. Corncob granules resemble fragments of corn, a food used by free-ranging house sparrows [13]. Thus, one might expect that house sparrows would intentionally ingest corncob granules, and, in fact, bird mortality has resulted from the use of this carrier type [22]. One reason why corncob granules may have presented less risk in our experiment could have been because they were not sought after as a food source by the birds.

Food in the form of mixed bird seed (which included cracked corn) was available to the house sparrows *ad libitum*.

The carrier type by pesticide load experiment also indicated that decreasing the pesticide load per granule could decrease avian risk, as was suggested by Best and Fischer [9]. If the pesticide load per granule was decreased, a bird may become satiated and stop ingesting granules before it consumed enough to cause adverse effects.

The size by color experiment suggested possible size effects, but the findings were inconclusive. The behavior data suggested that greater exposure to the pesticide occurred with the large granules, which represented the upper size range of grit used by this species [14]. The mortality data, however, indicated that greater exposure to the pesticide occurred with the small granules. We anticipated that color would have an influence on exposure because house sparrows have shown a preference for white grit particles and an avoidance of blue grit [7], but the findings from this experiment did not support this hypothesis.

Overall, we expected to see greater effects in both experiments. We wanted the magnitude of effect to be large enough to compare differences in granular formulations, therefore, conditions were manipulated to ensure high pesticide exposure. We did this by using application rates that greatly exceeded EPA's current level of concern (0.5 LD50/ft^2). In addition, by conducting the experiments in aviaries, we forced the birds to forage among the pesticide granules. There were no areas within the aviaries equivalent to untreated fields or edge habitats. Lastly, the aviaries were equipped with limited perches, which induced the birds to spend more time on the ground. All of these factors potentially increased the likelihood of pesticide exposure. Forcing the birds to forage in a confined area, however, could have potentially decreased pesticide exposure. The intensity of foraging activity seemed to accelerated the rate of incorporation of the pesticide granules, possibly making the granules less accessible to the birds than under more natural conditions. Counteracting this would be the fact that the soil in the aviaries was protected from direct rainfall, which would accelerate granule incorporation in the field [23]. It is important to note that our experiments were designed to compare differences among formulations, not to estimate the magnitude of effects under field conditions.

The Midwest region of the United States had unusually large amounts of rain in 1993, resulting in severe flooding throughout Iowa and elsewhere. It is possible that, despite our efforts to maximize bird exposure to the pesticide, the rain interfered with the effects in both experiments. We intended our experiments to be conducted on dry soil, thereby limiting the possible routes of exposure to those resulting from ingestion [9]. The unexpected frequency and intensity of the rainstorms (i.e., high winds resulting in wind-driven rain), however, made it impossible to keep the soil surface completely dry in some of the aviaries. During the carrier type by pesticide load experiment, up to 75% of the soil surface became wet in some of the aviaries. Although there was less rain during the size by color experiment, up to 50% of the soil surface became wet in some aviaries.

Soil wetness in the aviaries may have confounded the effects of pesticide load per granule. As the pesticide granules became wet, the likelihood of dermal exposure may have increased as the pesticide "washed off" the granules. This may have decreased the pesticide load effects because, although the number of granules/ft^2 varied, the amount of pesticide/ft^2 was the same and the amount of pesticide coming off the granules could have been similar for each treatment. Thus, birds may have been dermally exposed to the same amount of pesticide rather than to different loads, as intended, via ingestion.

Granular carrier type, size, and color effects also may have been diminished because of the heavy rains. For example, if the pesticide came off the granules, the granules would have been less toxic when they were ingested, making the relative differences in risk between carrier types less apparent. Carrier type effects also could have been diminished as the granules became wet because their appearance (e.g., surface texture) and integrity [12] may have been altered, thus changing their relative attractiveness to the birds. The effects of wet and dry soil conditions on avian risk from different formulations of granular pesticides are currently under investigation at our facility.

Another factor that may have interfered with our attempts to maximize exposure was the manner in which the birds were fed and the amount of feed given to them. By scattering the feed on the soil, we attempted to simulate natural feeding conditions by intermixing food and grit sources and by creating a situation where birds would forage among the granules. An *ad libitum* food supply was provided to ensure that there were no food deprivation effects. The highly visible and readily accessible food supply, however, may have decreased the likelihood of granule ingestion. The birds may have ignored the granules as a possible food source because there was no need to search for alternative foods. In addition, the abundant scattered feed may have made the granules less visible or created a visual distraction on the soil surface. If less food had been available, the exposure scenario may have been different. This also is under investigation.

In addition to the factors that may have diminished the effects of the four formulation variables, the effects that did occur may have been less evident because of the number of deaths during the experiments that were not related to pesticide exposure. Studies conducted in previous and subsequent years (with more "normal" rainfall), and using the same test system, resulted in little to no background mortality in the control groups, which indicates that the test system is appropriate. In 1993, the temperatures were below normal and the ground was continually wet, and it may be that the cool, wet conditions affected the general health of free-ranging house sparrows. Thus, using birds captured from a population that may have been stressed already could have resulted in stress-related deaths during the experiments.

EPA's use of the acute toxicity of a pesticide and the estimated number of granules exposed after application to assign comparative risks to birds does not take into account other factors that can influence avian exposure to granular pesticides. Our evaluation of different granular pesticide formulations indicates that there are at least two other factors besides those currently used by the EPA that can influence avian exposure to granular pesticides, namely, granular carrier type and pesticide load per granule. It is likely that other granule characteristics and factors such as weather, pesticide application techniques, and bird species differences also may be found to influence avian exposure to granular pesticides. Further research will help determine which factors influence exposure and the extent of their influence. With such information, it may be possible to more accurately estimate the risks that granular pesticides pose to birds and for industry to develop granular pesticide formulations that are less hazardous to birds.

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Table 1. The number of birds found dead for each treatment group (n=45) in the two experiments.

Carrier type by pesticide load experiment

	Silica	Clay	Corncob
Control	1	4	2
Low	2	4	2
Medium	2	5	2
High	8	3	4

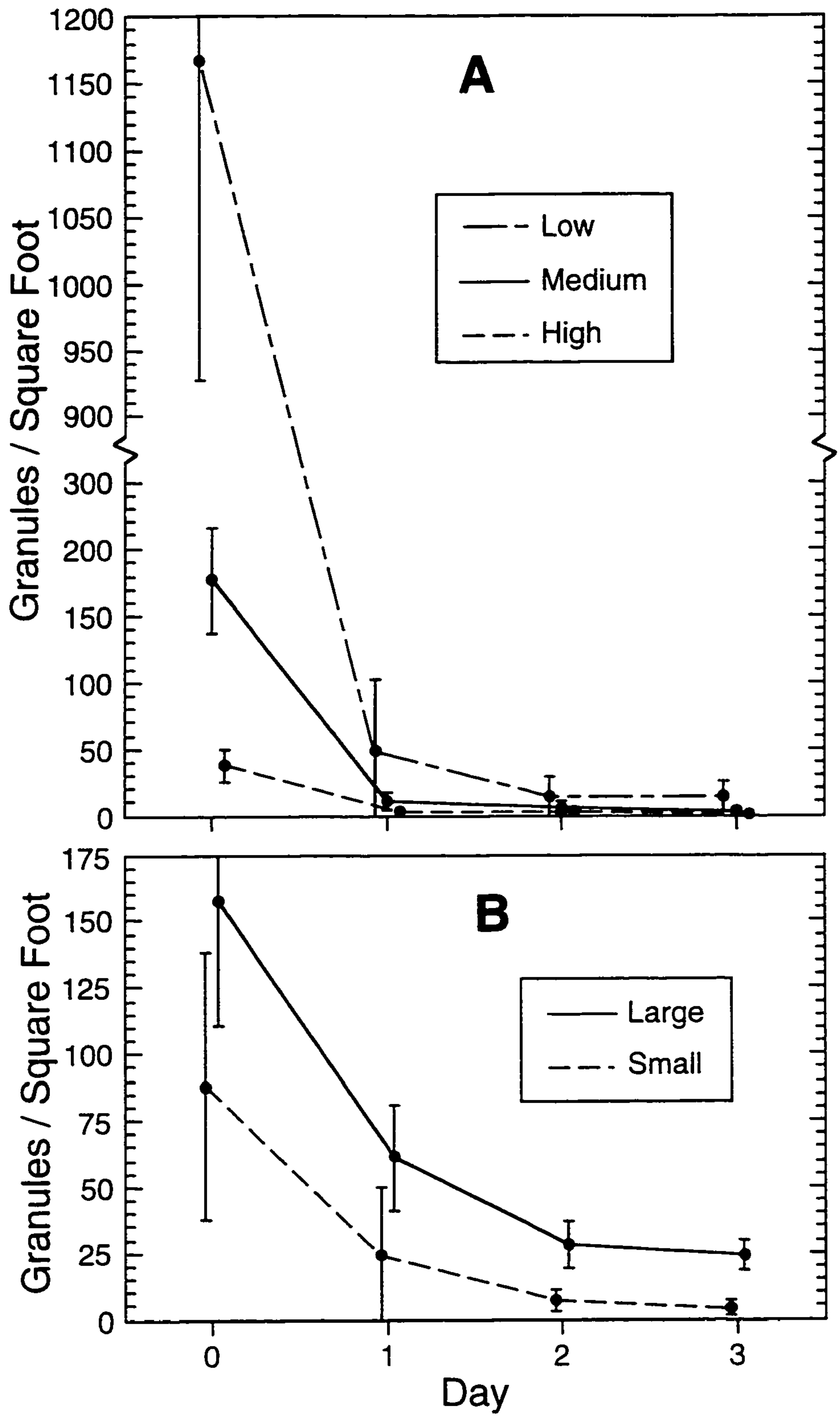
Granule size by color experiment

	Small	Large
Control	4	2
White	4	0
Blue	4	1

Figure Captions:

Fig. 1. Silica granule counts on the soil surface on days 0 to 3 of the carrier type by pesticide load (A) and size by color (B) experiments (three replicates).

Fig. 2. Plasma ChE activities (mean $\pm$ 1 S.E.), expressed as percentages of controls, for each carrier type for study days -2, 1, and 3 in the carrier type by pesticide load experiment (three replicates).


A
Low
Medium
High
Granules / Square Foot
1200
1150
1100
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1000
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B
Large
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Granules / Square Foot
175
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1
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Day

Figure 1

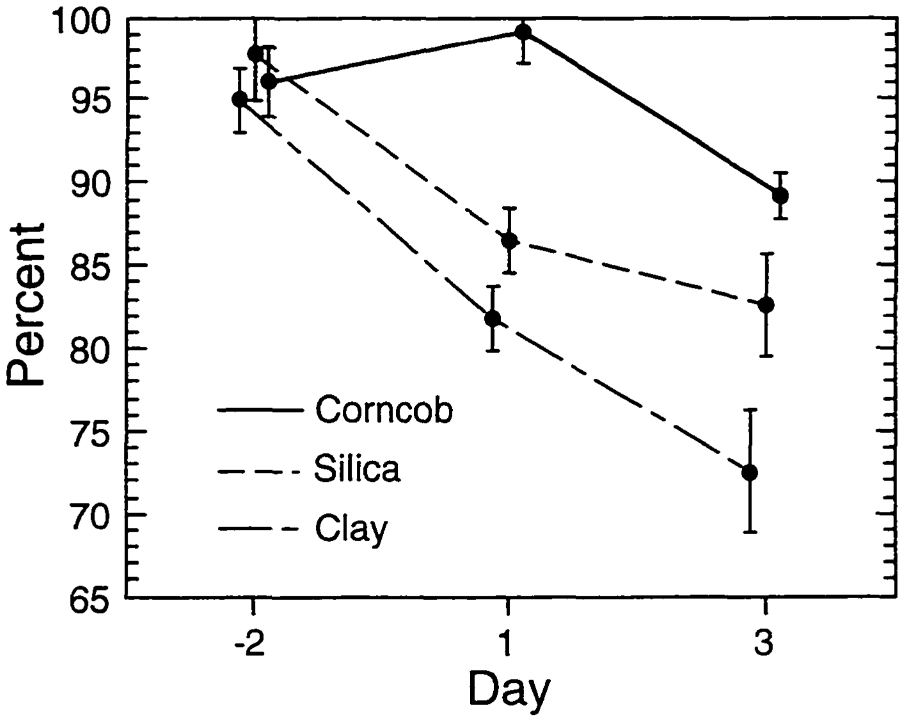

Figure 2

CHAPTER 3: EFFECTS OF GRANULAR PESTICIDE FORMULATIONS AND SOIL MOISTURE ON AVIAN EXPOSURE

A paper submitted to Environmental Toxicology and Chemistry

Tamara R. Stafford and Louis B. Best

Abstract–Two experiments were conducted to evaluate the influence of granular carrier type, pesticide load per granule, and soil moisture on avian risk of adverse effects from granular pesticides. Bird behavior, survival, and brain and plasma cholinesterase activity were used to assess exposure. In the first experiment, silica and corncob granules formulated with fensulfothion at either 1/2 or 1/32 of the LD50 were offered to captive house sparrows (*Passer domesticus*) in bands on dry soil. The corncob carrier and granules formulated with 1/2 the LD50 presented the greatest risk to birds. In the second experiment, fensulfothion was formulated on three carrier types (silica, clay, and corncob) and offered to house sparrows in bands on wet or dry soil. Overall, formulations with corncob as the carrier and dry-soil conditions presented the greatest risk to birds. Wet-soil conditions reduced the risk of adverse effects for all three granular carrier types, but the greatest risk reduction occurred with corncob and silica granules. Differences in adverse effects associated with various carrier types and soil moisture conditions can add complexity when assessing risk to birds from granular pesticides.

INTRODUCTION

Granular pesticides are used extensively throughout the United States to control pests in agricultural crops. Because many granular pesticides are highly toxic to birds [1,2] and are accessible to birds after pesticide application, there is concern over the risk that granular pesticides pose to birds [3,4]. The Environmental Protection Agency (EPA) currently evaluates potential risks of granular pesticides to birds on the basis of the acute avian toxicity of the compound and the number of granules exposed after application [5]. Physical characteristics of the granules (e.g., granular carrier type, pesticide load per granule, granule size, granule color), however, also may influence potential risks to birds. In addition, other factors could affect avian risk such as weather conditions, application rates and techniques, the number of applications per season, and bird species differences in foraging patterns and habitat use. Understanding which factors influence exposure, and the extent of their influence, is important in the development of a more comprehensive avian risk model.

To examine the influence that certain characteristics of granular pesticides might have on avian risk, two experiments were conducted in 1993 that evaluated the effects of carrier type, pesticide load per granule, granule size, and granule color [6]. The results from those experiments showed that both the carrier type and the pesticide load per granule influenced avian risk, although the magnitude of the effects was much less than expected (i.e., < 18% mortality in any treatment). The EPA currently considers avian risk to granular pesticides too great if pesticide applications result in more than 0.5 $LD50/ft^2$ [5]. Thus, greater mortality rates were expected in the 1993 experiments because we used application rates of 50 and 100 $LD50/ft^2$.

Two factors could have interfered with the results of the 1993 experiments: soil moisture and the amount of feed given to the test animals. Pesticide granules were applied in bands on a 1.5-cm deep soil surface inside roofed aviaries, and feed was scattered on the soil surface to provide *ad libitum* access. The experiments were designed to be conducted on a dry-soil surface, but Iowa had unusually large amounts of rain in 1993, and the frequency and intensity of the rainstorms made it impossible to keep the soil surface completely dry in many of the aviaries. As the granules on the soil surface became wet, the pesticide may have "washed off" the granules, making them less toxic and, consequently, decreasing the potential for risk from granule ingestion but possibly increasing the risk from dermal exposure and/or ingestion of contaminated feed. Because our experiments were designed to evaluate granule ingestion as the primary route of exposure [7], the loss of pesticide from the granules could have diminished the effects for the four variables we evaluated.

In the 1993 experiments the birds were given abundant amounts of feed to ensure that there would be no adverse effects from hunger stress, and the feed was scattered on the soil surface to encourage birds to forage among the granules. The highly visible and readily accessible food supply, however, may have decreased the likelihood of granule ingestion. For example, carrier types likely to be mistaken as food items may have been ignored by the birds as a possible food source because there was no need to search for alternative foods. In addition, the abundant scattered feed may have created a visual distraction on the soil surface. If less food had been available, the exposure scenario could have been different.

The present study consisted of two experiments: 1) a reevaluation of the effects of carrier type and pesticide load per granule on avian risk but under more normal weather conditions and 2) an evaluation of the influence of wet and dry soil on carrier type effects. Three commonly used granular pesticide carriers were selected for study: silica, corncob, and a heat-treated montmorillonite clay. These carrier types may be consumed by birds as grit or food or may be inadvertently ingested while birds forage [6,7]. Soil moisture was evaluated because the routes of exposure (e.g., ingestion or dermal) may be different under wet-soil versus dry-soil conditions [7]. In addition, soil moisture may alter granule appearance and integrity [8], thus changing the relative attractiveness of the granules to birds. Pesticide load per granule was evaluated because this determines the number of granules a bird would have to consume to be adversely affected. The amount of feed given to the test birds in both experiments was reduced from that given in the 1993 experiments. The reduced feed probably presented the test birds with a more realistic approximation of the amount of food free-ranging birds encounter. Our hope was that the information from these experiments would be useful in developing a more comprehensive avian risk model to more accurately estimate the risks that granular pesticides pose to birds.

METHODS

Study variables

Fensulfothion (*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate), an organophosphate pesticide, was used in this study because it is highly toxic to birds [1,9]. All pesticide formulations were provided by the Bayer Corporation (Kansas City, MO, USA). All three granular carrier types were formulated with a pesticide load of 1/2 the LD50 (LD50 = 0.3 mg/kg of technical grade fensulfothion [1]) per granule for a 27-g house sparrow (*Passer domesticus*). Silica and corncob granules also were formulated with a load of 1/32 the LD50. Hereafter, the loads will be referred to as high and low, respectively. Each carrier type was sieved to obtain granules 0.6 to 1.0 mm in diameter, the size of grit most frequently consumed by free-ranging house sparrows [10]. An analysis of the percentage active ingredient conducted by Bayer for each formulation verified that the actual concentrations fell within the range of 70-130% of the nominal concentration, which the EPA considers an acceptable analytical recovery [5].

Subjects and husbandry

The house sparrow was chosen as the experimental species for the reasons set forth in Best and Gionfriddo [8]. Test birds were captured in rural areas of Story County, IA, USA, by using mist nets. Captured birds were weighed, marked with a numbered leg band for identification, and acclimated in an outdoor holding facility for at least 7 d before being used in an experiment. A wild bird seed mix (Cardinal Brand Wild Bird Feed, Des Moines Feed Co., Des Moines, IA, USA) containing millet, milo, cracked corn, sunflower seeds, peanuts, and wheat was made available to the birds on an *ad libitum* basis, and the water (Ames municipal water) was supplemented with a vitamin and electrolyte mix (Vitamins & Electrolytes, SOLVAY Animal Health, Inc., Mendota Heights, MN, USA). The holding facility used for acclimatization was roofed (wood) and enclosed with hardware cloth. The facility contained at least two feeding stations and two water stations and was equipped with perches and sheltered areas.

Study design

The granular carrier type and pesticide load per granule were evaluated by using a completely randomized design with five treatments: corncob granule with high load, corncob granule with low load, silica granule with high load, silica granule with low load, and control (no granules). The control was designed to demonstrate that the stress of the test system did not adversely affect bird health. In 1993, the clay carrier and an intermediate pesticide load per granule (1/8 the LD50) had been included in the carrier type by pesticide load experiment [6]. These were excluded this time in an effort to increase the number of replicates so as to increase the power of the statistical tests. Silica and corncob were selected rather than clay because in the 1993 experiment the bird responses with clay were intermediate to those with silica and corncob. Treatments were randomly assigned to 5 study aviaries (the experimental unit), and 10 house sparrows (5 females, 5 males) were randomly assigned to each treatment. Because application rates of 50 and 100 LD50/ft^2 resulted in minimal effects in 1993, the application rate was increased to 250 LD50/ft^2 (500 or 8000 granules/ft^2). This experiment was replicated four times, and each replicate ran for 3 d.

The effects of granular carrier type and soil moisture on avian risk from granular pesticides were evaluated by using a 4 (carrier type: silica, clay, corncob, control [no granules]) x 2 (soil moisture: wet, dry) factorial design. Treatments were randomly assigned to 8 study aviaries and the number of birds per treatment and experiment duration were the same as for the carrier type and pesticide load experiment. An application rate of 200 LD50/ft^2 (400 granules/ft^2) and the high-load formulations for each of the carrier types were used in this experiment. This experiment was replicated three times.

Both experiments were conducted in roofed (corrugated steel) outdoor aviaries that measured 3.6 x 3.6 x 1.8 m and were enclosed with a combination of plexiglass and hardware cloth. Aviaries were equipped with perches, and flashing encircled the bottom 30 cm of each outside wall to prevent water from entering the aviary. Each aviary had a simulated soil surface at least 1.5 cm deep. Top soil was obtained from a site nearby and was screened to remove rocks and debris before being put in the aviaries. Maximum temperature and total precipitation were recorded daily at a weather station 12 km west of the aviaries [11]. The addition of flashing to the aviaries and a return to "normal" precipitation after 1993 made it possible to keep the soil dry.

All study aviaries were supplied with one water station (well away from the pesticide granules), and a maintenance diet of feed was scattered evenly on the soil surface. Through preliminary tests, an appropriate maintenance diet (i.e., the amount of feed necessary for the birds to survive without showing adverse effects) was determined to be 320 to 370 g of seed per aviary (1.0 to 1.5 kg of food was offered to the birds in the 1993 experiments). About 200 g of feed were scattered in each aviary 2 d before an experiment began (day -2), and another 120 to 170 g were scattered after pesticide application (day 0). Birds were weighed 2 d before pesticide application (day -2) and again 3 d after application (day 3), or when they were found dead, by using a 50-g Pesola scale.

The granular pesticide was applied in bands 7 in (18 cm) wide and 36 in (90 cm) apart, spacing similar to normal rowcrop applications. The pesticide bands were 3.6 m long and each aviary had four bands. Before pesticide application, each band was marked by running a nylon rope along each side of the band for the length of the band. The preweighed amount of pesticide was then distributed by hand to the band. To ensure even granule distribution, the pesticide for a particular band was divided into 3 portions and applied to 1.2-m-long sections of the band. The nylon ropes were removed immediately after pesticide application.

After pesticide application in the carrier type by soil moisture experiment, water was applied to the soil of the aviaries designated as the wet-soil treatments. A nozzle was attached to a garden hose, and the flow of water was set to simulate a light rain. As a result, the soil surface was relatively undisturbed, and the soil was thoroughly soaked (within 2 min) without puddling. Soil wetness was maintained by checking the aviaries twice daily, and water was reapplied as needed.

After pesticide application (and water application in the carrier type by soil moisture experiment), exposed granules were counted in one randomly selected square foot of one band within each aviary, and a count was made each day thereafter at the same location. In the carrier type by soil moisture experiment, granules were counted in all three replicates, whereas in the carrier type and pesticide load experiment, granule counts were made in two of the four replicates. Exposed granules were counted only in the silica treatments; the clay granules were difficult to distinguish from the soil and the corncob granules blended in with the scattered feed.

Responses measured

Pesticide exposure was assessed by observing bird behavior and mortality, and measuring plasma and brain cholinesterase (ChE) activity. After pesticide application, birds were observed twice daily (midmorning and late afternoon) for abnormal behaviors that might indicate pesticide exposure. Birds were observed for at least 1 min per aviary, and more time was spent when needed (i.e., many of the birds in an aviary were exhibiting symptoms). The behaviors included: ataxia (muscular incoordination), asthenia (weakness, debility), hyporeactivity (diminished reaction to stimuli), hyperexcitability (increased reaction to stimuli), pilorection (feathers held erect), catatonia (extreme unresponsiveness and inactivity), unkemptness (lack of preening), and wing drop (wing position abnormally low) [6,12]. Of the symptoms observed, only ataxia, hyporeactivity, and catatonia occurred with any regularity and, therefore, were the only symptoms analyzed. Any deaths occurring during the experiments were recorded, and carcasses were collected for brain ChE analysis.

Birds were captured within the aviaries using hand-held nets so that blood samples could be obtained from the birds. When netting the birds, technicians stood between the pesticide bands so as not to disturb the granules within the bands. A blood sample (about 40 μL) was collected from each bird on days -2, 1, and 3 (except when a bird died during the experiment). from the ulnaris (wing) vein into a heparinized capillary tube and kept on wet ice until centrifugation. Blood samples were centrifuged at 7500 RPMs for 20 min, and the plasma was stored at -70 C until analyzed.

All birds surviving to the end of an experiment were euthanized (CO_2 asphyxiation) for brain ChE analysis and frozen at -10 C until the brains were analyzed. Frozen whole brains were excised and homogenized in 0.1M Tris buffer (pH 7.4) at a 200-fold dilution by using a Tri-R-Stir® homogenizer (Rock Centre, NY, USA) at 45% power for 3 strokes (15 s) and at 60% power for another 6 strokes (20 s). The homogenate was kept on wet ice until assayed (within 2 h).

ChE analysis

Plasma and brain ChE analyses were used to assess ChE inhibition resulting from exposure to the pesticide. Analyses were conducted at Iowa State University according to the methods of Ellman et al. [13], modified for use on a Vmax 96-well plate reader (Molecular Devices Corporation, Palo Alto, CA, USA). Reagent volumes used in the ChE assays were 10 μL 0.042 M acetylthiocholine iodide (ATCI) as substrate and 20 μL 0.004 M 5,5-dithio-bis-(2-nitrobenzoic acid) in 250 μL of 0.1M Tris buffer (pH 8.0). Reagents and buffers were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The sample volume for both plasma and brain ChE analyses was 5 μL. Assays were conducted at 25 C and a wavelength of 405 nm. For plasma samples, 15 absorbance readings were taken during a 2-min period. For brain samples, 20 absorbance readings were taken during a 3-min period. Three subsamples of each plasma and brain sample were analyzed, and additional assays were conducted if the coefficient of variation for the three subsamples was more than 15%. Plasma ChE activity was expressed in International Units (U/L) and represented the amount of ChE catalyzing the transformation of 1 μmol of substrate/min/L. Brain ChE activity was expressed as μmoles of substrate (ATCI) hydrolyzed/min/g tissue.

Data analysis

Because no significant sex differences were found for any of the dependent variables, the data from males and females were combined. Aviary was the experimental unit in all analyses. Body weights were analyzed by using repeated measures analysis of variance (ANOVA), with measurement day (days -2 and 3) as the repeated measures factor. Because plasma ChE activity was measured on 3 separate days (i.e., days -2, 1, and 3), these data were analyzed by using multivariate analysis of variance (MANOVA) [14]. Brain ChE activity, mortality rates, and behavioral symptom frequencies were analyzed by using two-way ANOVA. An alpha level of 0.05 was used for all statistical tests. Fisher's Least Significant Difference test was used to make post-hoc pairwise comparisons among treatments at the 0.05 significance level.

RESULTS

Carrier type and pesticide load experiment

The initial granules counts (Fig. 1a) taken immediately after application were much lower than the number of granules applied per square foot because many of the granules were immediately lost into the pores and crevices of the soil surface. Silica granules were rapidly incorporated into the soil after application due primarily to bird foraging activities (scratching and probing). Although the degree of incorporation of the silica granules may have differed from that of the corncob granules, we are confident that the corncob granules also were incorporated into the soil. The proportion of granules incorporated seemed to be the same regardless of the number of granules applied to the soil surface (compare the high and low curves, Fig. 1a). A balance may have been reached such that bird foraging activities brought as many granules up to the surface as were buried. A similar situation might occur in the field, but we suspect that fewer granules would be available after incorporation because there would be a deeper mixing zone in the field. In our test system, the soil was only 1.5 cm deep on a concrete surface.

Treatment condition significantly influenced bird survival ($F[4, 15] = 17.00$, $p < 0.01$; Table 1). More birds died when exposed to granules with a high pesticide load than when exposed to granules with a low pesticide load. In addition, more birds died when exposed to the corncob-high treatment than when exposed to the silica-high treatment. Dead birds were mostly found on days 0 and 1 of the experiments.

Treatment condition significantly influenced brain ChE activity ($F[4, 15] = 10.32$, $p < 0.01$; Table 1). Brain ChE activity was significantly lower in birds exposed to corncob granules with a high pesticide load than in birds exposed to granules (silica or corncob) with a low pesticide load. Brain ChE activity also was significantly lower in birds exposed to silica granules with a high pesticide load than in birds exposed to silica granules with a low pesticide load. No significant interactions were found between carrier type and pesticide load.

In the analysis of plasma ChE activity, significant main effects were found for treatment and day of measurement ($F[4, 15] = 8.35$, $p < 0.01$; $F[2, 14] = 173.86$, $p < 0.01$, respectively). These main effects, however, were qualified by a significant interaction between treatment and day of measurement ($F[8, 28] = 7.96$, $p < 0.01$; Table 1). As expected, treatment did not significantly influence plasma ChE activity on day -2 ($F[4, 15] = 2.29$, $p = 0.2$). Treatment did, however, significantly influence plasma ChE activity on day 1 ($F[4, 15] = 23.33$, $p < 0.01$). Day 1 plasma ChE was lower in birds exposed to corncob granules than in control birds or in birds exposed to silica granules with a low pesticide load. Day 1 plasma ChE also was lower in birds exposed to silica granules than in the control birds. Treatment also tended to influence plasma ChE activity on day 3 ($F[4, 15] = 2.79$, $p = 0.07$). Day 3 plasma ChE was significantly lower in birds exposed to silica granules or to corncob granules with a low pesticide load than in the control birds. Day 3 plasma ChE activity of birds exposed to the corncob-high treatment was not significantly lower due to the high mortality and decreased the statistical strength in that condition.

Several abnormal behaviors were seen in all treatments (including controls) such as ataxia, asthenia, hyporeactivity, pilorection, catatonia, and wing drop, but only hyporeactivity, catatonia, and ataxia occurred with any regularity. Treatment significantly influenced the frequency of hyporeactive behavior ($F[4, 15] = 6.68$, $p < 0.01$; Table 1). Hyporeactivity occurred more frequently in birds exposed to silica- and corncob-high granules than in the control birds . Hyporeactivity also occurred more frequently in birds exposed to silica-high granules than in birds exposed to corncob- or silica-low granules. Treatment also significantly influenced the frequency of catatonia ($F[4, 15] = 3.48$, $p = 0.04$; Table 1). Catatonia occurred more frequently in birds exposed to corncob-high granules than in birds exposed to control granules or to corncob- or silica-low granules. Treatment did not significantly influence ataxia ($F[4, 15] = 1.83$, $p = 0.2$). In the analysis of body weights, no significant effects were found.

Carrier type by soil moisture experiment

As with the carrier type and pesticide load experiment, silica granules were rapidly incorporated into the soil after application (Fig. 1b). Incorporation of the granules into the wet soil occurred when water was applied to the soil on day 0; incorporation into the dry soil resulted mainly from bird foraging activities. Despite the rapid incorporation of the granules, at least 3 LD50/ft^2 were available to the birds on the soil surface in all silica treatments throughout the experiment.

Both carrier type and soil moisture significantly influenced bird survival ($F[3, 16] = 5.64$, $p < 0.01$; $F[1, 16] = 8.02$, $p = 0.02$, respectively; Table 2). These main effects, however, were qualified by a nearly significant carrier type by soil moisture interaction ($F[3, 16] = 2.49$, $p = 0.10$). More birds died when exposed to corncob granules on dry soil than did birds in the other treatments. More birds also died when exposed to clay granules on dry soil than when exposed to silica granules on wet soil. As with the carrier type and pesticide load experiment, dead birds were mostly found on days 0 and 1.

In the analysis of brain ChE activity, significant main effects were found for carrier type and soil moisture ($F[3, 16] = 16.86$, $p < 0.01$; $F[1, 16] = 21.91$, $p < 0.01$, respectively; Table 2). These main effects, however, were qualified by a nearly significant interaction between carrier type and soil moisture ($F[3, 16] = 2.58$, $p = 0.10$). Brain ChE activity was significantly lower in birds exposed to corncob granules on dry soil than in birds exposed to corncob, silica, or clay granules on wet soil. Brain ChE activity was significantly lower in birds exposed to clay granules on dry soil than in birds exposed to corncob or silica granules on wet soil, and also was significantly lower in birds exposed to silica granules on dry soil than in birds exposed to silica granules on wet soil.

In the analysis of plasma ChE activity, significant main effects were found for carrier type and day of measurement of plasma ChE activity ($F[3, 16] = 15.80$, $p < 0.01$; $F[2, 15] = 58.04$, $p < 0.01$, respectively; Table 2). These main effects, however, were qualified by a significant interaction between carrier type and day of measurement ($F[6, 30] = 3.36$, $p = 0.02$). As expected, carrier type did not significantly influence plasma ChE activity on day -2 ($F[3, 16] = 0.18$, $p = 0.91$). Carrier type did, however, have a significant effect on plasma ChE on days 1 and 3 ($F[3, 16] = 11.92$, $p < 0.01$; $F[3, 16] = 14.58$, $p < 0.01$, respectively). On both days, plasma ChE was lower in birds exposed to treated granules than in control birds. No significant interactions were found between carrier type and soil moisture for any day.

Several abnormal behaviors were observed in all treatments, but only ataxia and hyporeactivity occurred with any regularity. The frequency of ataxia was influenced by carrier type ($F[3, 16] = 6.21$, $p < 0.01$; Table 2). Ataxia occurred more frequently when birds were exposed to clay granules than when they were exposed to silica granules. The frequency of hyporeactivity was influenced by soil moisture ($F[1, 16] = 7.58$, $p = 0.02$; Table 2).

Hyporeactivity occurred more frequently in the dry-soil treatments than in wet-soil treatments. No significant interactions were found between carrier type and soil moisture.

In the analysis of body weights, a significant main effect was found for day of measurement on body weights ($F[1, 16] = 233.43$, $p < 0.01$). This main effect, however, was qualified by a significant interaction between moisture and day of measurement ($F[1, 16] = 19.71$, $p < 0.01$). As expected, moisture had no significant effect on day -2 body weights, ($F[1, 16] = 0.05$, $p = 0.9$), indicating that random assignment of birds to dry and wet moisture treatments was successful. On day 3, birds in the wet-soil aviaries weighed significantly less ($\bar{X} = 25.3 \pm 0.4$g) than did birds in the dry-soil aviaries ($\bar{X} = 26.0 \pm 0.4$g; $F[1, 3] = 7.67$, $p = 0.02$). Because birds in the wet-soil treatments were often seen with soil caked on their feet, we suspect that the wet soil interfered with foraging activities, which could account for the decrease in body weight. Because no carrier type effect on body weights approached significance, it is doubtful that weight loss was due to pesticide exposure.

DISCUSSION

Our results demonstrated that adverse effects to birds from granular pesticides may be influenced by the type of carrier. Overall, corncob granules posed a greater hazard to house sparrows than silica or clay granules, possibly because the birds had a greater preference for corncob granules compared with silica or clay granules. This result was somewhat unexpected because previous research [6,15] had indicated that silica and clay granules were most preferred by house sparrows, whereas corncob granules were among the least preferred granule types.

We suspect that a stronger preference for the corncob granules in both the carrier type and pesticide load experiment and the carrier type by soil moisture experiment was due to the change in the amount of food given to the birds. In previous studies [6,15], commercial bird seed was available to the house sparrows, before and during the experiments, in quantities that far exceeded their food requirements. In the current experiments, a smaller amount of food was given to the birds. This may have stimulated the birds to explore other potential food sources within the aviary, thus increasing their potential to pick up corncob granules, particles similar to corn, a food used by free-ranging house sparrows [16]. Availability of food, therefore, may be a factor that could influence avian ingestion of pesticide granules. Although not measured in the current experiments, the birds perception of the different granule carrier types (i.e., as grit or food) could affect the relative importance that each carrier has on avian risk because carrier type probably influences both the intentional ingestion of a carrier type and the number of granules that would be consumed.

Pesticide load per granule also influenced the potential for adverse effects to birds. Exposure to granules with a high pesticide load resulted in greater adverse effects to birds than exposure to granules with a low pesticide load. A greater number of granules were available to the birds in the low-load treatments, but the number of granules required to cause an adverse effect in a bird may have exceeded the number of granules that the birds were likely to pick up, based on their grit/food consumption rate [7, 17]. Thus, lower toxic loads per granule may reduce the risk that granules pose to birds and may be an important means for risk mitigation.

The amount of moisture in the soil also influenced adverse effects from pesticide granules. In general, granules on wet soil posed less hazard to the birds than granules on dry soil. One possible explanation for this is that the pesticide on the granules on wet soil may have been released (i.e., washed off) when water was applied to the soil; therefore, the granules were less toxic when ingested. It is interesting that wet-soil conditions reduced adverse effects because it seemed likely that the opportunity for dermal absorption of the pesticide through the birds' feet as they foraged would have been enhanced if the pesticide was released from the carriers. Although dermal exposure may be of primary importance for pesticides applied as foliar sprays [18], little is known about the threat of dermal exposure to birds from granular pesticides. Our study was not designed to assess dermal exposure and, thus, provides no definitive answers to this question. But it does indicate that dermal exposure, in the absence of puddling, may not be a primary route of exposure for some pesticide formulations, which is consistent with the idea that ingestion presents the greatest risk to birds from granular pesticides [3].

Wet soil did not, however, reduce the adverse effects to test birds to the same extent for all three carrier types. Adverse effects from corncob and silica granules were significantly reduced on wet soil, whereas adverse effects from clay granules were not. Because carrier compositions differ [15], it follows that carrier types may influence exposure differently under various soil moisture conditions. For example, the pesticide may be released from the granules at different rates for different carrier types. It is probable that the release rates for silica, clay, and corncob carriers differ because the pesticide cannot absorb into the silica (quartz) granules (the pesticide is affixed with a water soluble "sticker"), but it can absorb into clay (lattice structure) and corncob (organic) granules [7]. As a result, silica granules may have released the chemical more quickly under wet conditions. If the birds had ingested the granules, they may have had little or no pesticide on them, thus posing less of a hazard on wet soil. Conversely, the other two carriers may have retained more of the pesticide under wet conditions, thus posing a greater hazard to the birds.

The particle weight of the different carrier types also may have contributed to the interaction between carrier type and soil moisture. The mean granule weights for our formulations of silica, clay, and corncob were 0.802, 0.367, and 0.092 mg, respectively. After water was applied to the soil, silica granules may have settled into the soil more readily than those of clay and corncob because silica granules are more dense. As a result, silica granules would have been less accessible to the birds than clay or corncob granules.

Because ingestion of granular pesticides is a primary route of exposure, conditions that are optimal for ingestion will pose the greatest risk to birds. Based on the results of our study, two factors that can influence the likelihood of ingestion are carrier type and soil moisture. Carrier type can influence ingestion because the various granule types used as pesticide carriers differ in composition, shape, surface texture, size, and color [15,19], which can influence the attractiveness of a given carrier to birds (whether the granule is perceived as grit or food). Soil moisture also may influence ingestion because rainfall can influence pesticide release from a granule and granule availability after rainfall. In addition, exposure to moisture can affect the integrity of some carrier types [8]. This may limit the amount of time they are intact and can be perceived as food or grit, thus decreasing the likelihood of ingestion [7].

Weather conditions also may influence avian foraging activities, grit consumption rates, and food availability. Foraging activities and grit consumption can be greater under cooler, wetter conditions [15,20], and consumption of pesticide granules, therefore, might be greater with cooler temperatures and precipitation. It may be that silica granules (the carrier type most likely to be consumed as grit) posed a greater hazard for house sparrows than did corncob granules in the 1993 experiments because the heavy rainfall and cooler temperatures that year probably resulted in increased grit consumption rates. Furthermore, during years when weather conditions are less favorable for birds and their food supply is affected, birds may be more likely to explore alternative food sources, which also may increase consumption of granules, such as corncob, that may be mistaken for food.

Differences in the adverse effects associated with various carrier types and weather conditions create complexity when attempting to predict risk to birds from granular pesticides or when trying to formulate products that will lessen risk. In our study, the hazard presented by the clay carrier seemed the most consistent under different soil moisture conditions compared with the hazard from the silica or corncob carriers. Ideally, carrier types should be selected for use that not only pose the least adverse effects to birds but that also vary little in risk from one environmental condition to another.

Because carrier type, pesticide load per granule, and soil moisture can influence avian risk from granular pesticides, both pesticide formulations and weather conditions should be considered when assessing avian risk from granular pesticides. With the inclusion of these factors in a risk exposure model, the risk of adverse effects from granular pesticides can be more accurately predicted. In addition, it is possible that other factors, such as application rates and bird species differences, might also influence the risk to birds posed by granular pesticides. Further research will help determine which factors influence exposure and the extent of their influence.

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Table 1. Means[a] (± 1 standard error) for each parameter in the carrier type and pesticide load experiment.

		Silica		Corncob	
	Control	Low	High	Low	High
Mortality	0.0a[b] (0.0)	0.0a (0.0)	2.0b (0.9)	0.0a (0.0)	6.0c (1.1)
Brain ChE Activity (μmol/min/g tissue)	37.1d (1.3)	33.5cd (1.2)	23.6ab (2.7)	28.1bc (2.5)	19.8a (2.6)
Plasma ChE Activity (μmol/min/L)					
Day -2	986 (22.5)	973 (31.4)	1016 (15.0)	996 (13.1)	1053 (15.7)
Day 1	864c (18.6)	575b (9.3)	482ab (38.9)	453a (42.2)	430a (55.4)
Day 3	841b (67.2)	543a (130.7)	524a (111.9)	421a (111.5)	683ab (31.2)
Behavior					
Hyporeactivity	0.0a (0.0)	0.5ab (0.3)	2.8c (0.6)	1.0ab (0.4)	1.8bc (0.5)
Catatonia	0.0a (0.0)	0.0a (0.0)	0.8ab (0.5)	0.0a (0.0)	1.5b (0.6)
Ataxia	0.0 (0.0)	0.0 (0.0)	1.0 (0.4)	0.3 (0.3)	0.5 (0.5)

[a]Each mean represents the average of four replicates with 10 birds in each aviary.
[b]Fischer's Least Significance Difference test was used to make within-row comparisons when the results of the ANOVA or MANOVA were significant and an interaction between carrier type and pesticide load was found. Means having the same letter are not significantly different at the 0.05 level.

Table 2. Means[a] (± 1 standard error) for each parameter in the carrier type by soil moisture experiment.

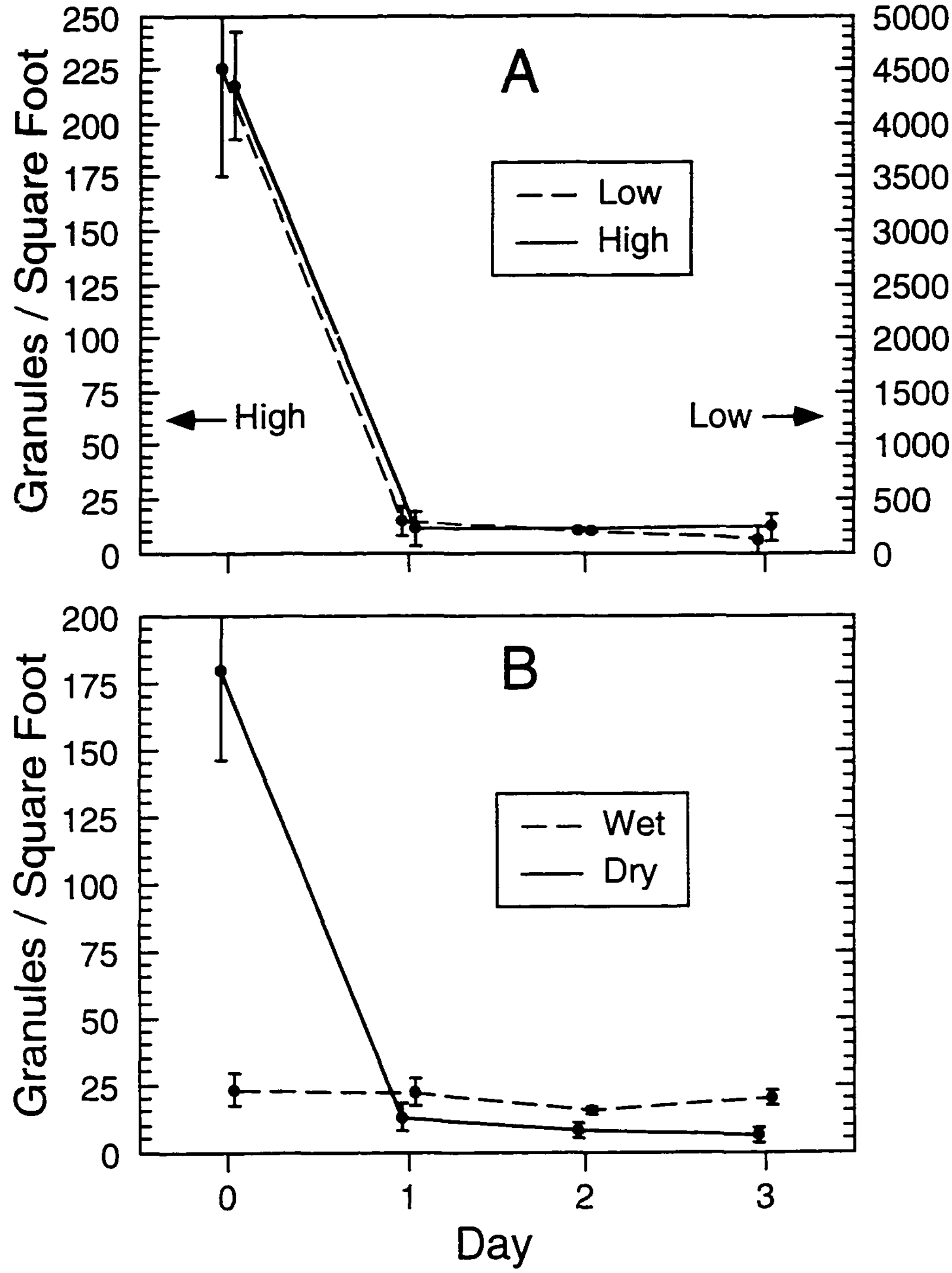
	Control		Silica		Clay		Corncob	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Mortality	0.3ab[b] (0.3)	1.0ab (1.0)	2.3abc (0.9)	0.0a (0.0)	2.7b (1.3)	1.3abc (0.9)	6.0d (1.2)	2.0abc (0.6)
Brain ChE Activity (μmol/min/g tissue)	36.8e (1.2)	37.6e (2.7)	21.0abc (1.4)	33.6de (1.3)	19.7ab (3.8)	26.5bcd (2.1)	15.1a (3.9)	27.4cd (1.2)
Plasma ChE Activity (μmol/min/L)								
Day -2	940 (59.7)	1024 (81.1)	949 (55.4)	955 (34.7)	1035 (18.2)	937 (19.4)	975 (71.5)	1001 (71.9)
Day 1	918 (42.3)	838 (4.9)	409 (8.6)	581 (125.4)	435 (92.7)	577 (106.6)	511 (104.2)	308 (99.6)
Day 3	897 (38.1)	878 (6.0)	659 (76.4)	601 (76.1)	662 (13.0)	525 (38.5)	650 (71.2)	596 (27.2)
Behavior								
Ataxia	0.0 (0.0)	0.0 (0.0)	0.3 (0.3)	0.0 (0.0)	2.0 (0.6)	0.3 (0.3)	0.7 (0.7)	0.7 (0.3)
Hyporeactivity	0.3 (0.3)	0.3 (0.3)	1.7 (0.3)	1.0 (0.0)	2.7 (0.3)	0.7 (0.3)	1.7 (1.2)	0.3 (0.3)

[a]Each mean represents the average of three replicates with 10 birds in each aviary.

[b]Fischer's Least Significance Difference test was used to make within-row comparisons when the results of the ANOVA or MANOVA were significant and an interaction between carrier type and soil moisture was found. Means having the same letter are not significantly different at the 0.05 level.

Figure Captions:

Fig. 1. Mean ($\pm$ 1 standard error) silica granule counts on the soil surface on days 0 to 3 of (A) the carrier type and pesticide load experiment (n=2 replicates), and (B) the carrier type by soil moisture experiment (n=3 replicates).



A
Low
High
Granules / Square Foot
250
225
200
175
150
125
100
75
50
25
0
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
High
Low
B
Wet
Dry
Granules / Square Foot
200
175
150
125
100
75
50
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0
1
2
3
Day

Figure 1

CHAPTER 4: EFFECTS OF APPLICATION RATE ON AVIAN RISK FROM GRANULAR PESTICIDES

A paper to be submitted to Environmental Toxicology and Chemistry

Tamara R. Stafford and Louis B. Best

Abstract–Fensulfothion was formulated on silica granules to evaluate the relationship between avian risk of adverse effects from granular pesticides and granule availability. Captive house sparrows (*Passer domesticus*) were offered the granular pesticide in bands on soil surfaces at application rates of 50, 100, 200, 400, 800, 1200, 1600, or 2000 LD50/ft^2. Individual pesticide granules were formulated with 1/2 LD50 for house sparrows (0.3 mg/kg). Bird behavior, survival, and brain cholinesterase activity were used to assess pesticide exposure. A significant negative correlation between brain ChE activity and the natural logarithm of application rate indicated that the relationship between avian risk and granule availability was asymptotic rather than linear.

INTRODUCTION

Granular pesticides are used extensively in the United States to control pests in agricultural crops and are typically applied to the soil surface in a band or in the seed furrow. Because many granular pesticides are highly toxic to birds [1,2], many pesticide labels require incorporation of the granules into the soil during application. Despite efforts to bury the granules, many remain exposed on the soil surface after application [3,4]. Because of the accessibility of the granules after application, regulatory authorities have expressed concern over the risk that granular pesticides pose to birds [5,6]. Currently, the Environmental Protection Agency (EPA) generates an index for avian risk based on the acute toxicity of a pesticide and the amount of toxicant exposed after application, which is expressed as LD50/ft^2 [6].

Implicit in the LD50/ft^2 risk index is the assumption that the potential risk to birds from granular pesticides increases as granule availability increases [6]. The EPA recognizes that there is uncertainty in the predictive value of the risk index because the relationship between avian risk and granule availability is not necessarily linear. It may be that the relationship is asymptotic and, at some point, avian consumption of the granules would level off even with further increases in granule availability [7]. If this were the case, the risk index may overestimate the risk for a granular pesticide that has a large number of granules exposed after application. This would be particularly pertinent for granular pesticides with a low, sublethal pesticide load per granule because birds could reach an asymptote in granule consumption before consuming enough granules to cause an adverse effect [8]. Increases in the application rates and availability of silica granules applied to cornfields did not seem to cause an increase in the consumption rates of the granules by birds [9] thus indicating that an assumption of linearity may be inappropriate. The main objective of our study was to evaluate the relationship between avian risk from a granular pesticide and granule availability.

METHODS

Study design

Silica (quartz) granules were selected as the granular carrier for this experiment because free-ranging birds consume similar, naturally occurring particles as grit [10,11], and house sparrows (*Passer domesticus*) have demonstrated a preference for silica granules over five other granular carriers in laboratory trials [12]. Granules were formulated by the Bayer Corporation (Kansas City, MO, USA) with fensulfothion, an organophosphate pesticide that is highly toxic to birds [1,13]. The pesticide load per granule was 1/2 the LD50 for a 27-g house sparrow (LD50 = 0.3 mg/kg of technical grade fensulfothion [1]). An analysis of the percent active ingredient conducted by the Bayer Corporation verified that the actual concentration was

93% of the nominal concentration, which the EPA considers an acceptable analytical recovery [14].

The test birds, house sparrows, were captured in rural areas of Story County, Iowa by using mist nets. Captured birds were weighed, marked with a numbered leg band, and acclimated to captivity for at least 7 d before being used in the study. A wild bird seed mix (Cardinal Brand Wild Bird Feed, Des Moines Feed Co., Des Moines, IA, USA) containing millet, milo, cracked corn, sunflower seeds, peanuts, and wheat was available to the birds *ad libitum*, and the water (Ames municipal water) was supplemented with a vitamin and electrolyte mix (Vitamins & Electrolytes, SOLVAY Animal Health, Inc., Mendota Heights, MN, USA).

The effect of application rate on avian exposure to pesticide granules was evaluated by using 8 pesticide application rates: 50, 100, 200, 400, 800, 1200, 1600, and 2000 LD50/ft^2, resulting in granule application rates of about 100, 200, 400, 1600, 2400, 3200, and 4000 granules/ft^2, respectively. In addition, control birds were exposed to blank silica granules (no pesticide) applied at a rate of about 400 granules/ft^2. Treatments (including controls) were randomly assigned to 9 study aviaries, and 8 house sparrows (4 females, 4 males) were randomly assigned to each treatment. The experiment was replicated two times, and each replicate ran for 3 d.

The experiment was conducted in roofed (corrugated steel) outdoor aviaries that measured 3.6 x 3.6 x 1.8 m. Each aviary had a simulated soil surface at least 1.5 cm deep. Top soil was obtained from a site nearby and was screened to remove rocks and debris before being put in the aviaries. Each aviary was supplied with one water station, and bird seed was scattered evenly on the soil surface of each aviary. About 200 g of feed were scattered in each aviary 2 d before exposure (day -2), and another 120 to 170 g were scattered after pesticide application (day 0). Birds were weighed 2 d before pesticide application (day -2) and again 3 d after application (day 3), or when they were found dead, by using a 50-g Pesola scale.

The granular pesticide was applied in bands 7 in (18 cm) wide and 36 in (90 cm) apart, similar to normal rowcrop applications. The pesticide bands were 3.6 m long and each aviary had 4 bands. Before pesticide application, each band was marked by running nylon ropes along each side of the band for the length of the band. The preweighed amount of pesticide was then distributed by hand to the band. To ensure even granule distribution, the pesticide for a particular band was divided into 3 portions and applied to 1.2-m-long sections of the band. The ropes were removed immediately after pesticide application.

Pesticide exposure was assessed by recording bird behavior, mortality, and brain cholinesterase (ChE) activity. After pesticide application, birds were observed twice daily (midmorning and late afternoon) for abnormal behaviors that might indicate pesticide exposure. Birds were observed for at least 1 min per aviary, and more time was spent when needed (i.e., many of the birds in an aviary were exhibiting symptoms). The behaviors included: ataxia (muscular incoordination), asthenia (weakness, debility), hyporeactivity (diminished reaction to stimuli), hyperexcitability (increased reaction to stimuli), pilorection (feathers held erect), catatonia (extreme unresponsiveness and inactivity), unkemptness (lack of preening), and wing drop (wing position abnormally low) [8,15]. Of the symptoms observed, only ataxia, hyporeactivity, and catatonia occurred with any regularity and, therefore, were the only symptoms analyzed. Any deaths occurring during the experiment were recorded, and carcasses were collected for brain ChE analysis.

Cholinesterase analysis

All birds surviving to the end of an experimental replicate were euthanized for brain ChE analysis and frozen at -10 C until the brains were analyzed. Frozen whole brains were excised and homogenized in 0.1M Tris buffer (pH 7.4) at a 200-fold dilution by using a Tri-R-Stir® homogenizer (Rock Centre, NY, USA) at 45% power for 3 strokes (15 s) and at 60% power for another 6 strokes (20 s). The homogenate was kept on wet ice until assayed (within 2 h).

Brain ChE analysis was conducted at Iowa State University according to the methods of Ellman et al. [16], modified for use on a Vmax 96-well plate reader (Molecular Devices Corporation, Palo Alto, CA, USA). Reagent volumes used in the ChE assays were 10 μL 0.042 M acetylthiocholine iodide (ATCI) as substrate, and 20 μL 0.004 M 5,5-dithio-bis-(2-nitrobenzoic acid) in 250 μL of 0.1M Tris buffer (pH 8.0). Reagents and buffers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The sample volume was 5 μL. Assays were conducted at 25 C and at a wavelength of 405 nm. Twenty absorbance readings were taken during a 3-min period. Three subsamples of each brain sample were analyzed and additional assays were conducted if the coefficient of variation for the 3 subsamples was more than 15%. Brain ChE activity was expressed as μmoles of substrate (ATCI) hydrolyzed/min/g tissue.

Data analysis

Because the experiment contained only two replicates, application rate was treated as a continuous variable and regression analysis was used to evaluate all response measures. Brain ChE activity, mortality, and behavioral symptoms were analyzed by using simple linear regression analysis. Because an asymptotic relationship between application rate and the various dependent variables was a possibility, separate regression analyses were conducted using either application rate or a natural logarithm transformation of the application rate as the independent variable. A significance level of 0.05 was used for all statistical tests.

RESULTS AND DISCUSSION

For each dependent variable, the proportion of variation explained in the regression analysis (i.e., r^2) was greater when the natural logarithm of application rate was used as the independent variable than when the application rate was untransformed. This confirmed that the relationship between avian risk and granule availability was asymptotic. The negative linear relationship between the natural logarithm of the application rate and brain ChE activity was statistically significant ($\underline{t}[14] = -5.06$, $r^2 = 0.65$, $\underline{p} < 0.01$; Fig. 1). In each aviary to which the pesticide was applied, an average of 6 to 18% of the birds was found dead (Table 1), but no significant relationship was found between application rate and mortality. That a closer relationship was detected with brain ChE activity than with mortality was not unexpected considering that brain ChE activity is a continuous and, therefore, a more sensitive measure. Some birds showed signs of ataxia, catatonia, and hyporeactivity (Table 1), but no significant relationship was found between application rate and any behavioral symptom.

We were surprised that the birds did not show greater adverse effects from the granular pesticide for several reasons. The toxic load per granule was quite high, with each granule representing 1/2 of the LD50; thus, 1/2 the test population should have been killed if each bird had ingested at least 2 granules. The current risk model assumes that avian exposure to a pesticide will be about equal to the number of granules available per square foot [6]. If that were the case, however, no birds should have survived the test. In addition, the application rates we used were 100 to 4000 times greater than the EPA's current regulatory criteria (0.5 LD50/ft^2) for an unacceptable risk to birds [14]. Further, our test system probably represented a worst case scenario. For example, house sparrows generally have relatively high grit consumption rates [17], and they have demonstrated a preference for silica granules [12]. Lastly, house sparrows are sensitive to fensulfothion as indicated by a low LD50 (0.3 mg/kg), and the birds, being confined in aviaries, were forced to forage among the pesticide granules.

The asymptotic relationship found between brain ChE activity and silica granule availability seems to demonstrate that an assumption of linearity between avian risk and granule availability is inappropriate. That an asymptote was reached suggests that the current LD50/ft^2 risk index may be misleading in predicting avian risk because the index could predict excessively high levels of risk for granular pesticides that have a greater number of granules exposed per unit area after application. For example, the estimated number of exposed granules/ft^2 following application of 14 granular pesticide formulations, considered by EPA to pose the greatest risk to birds, ranged from 295 to 14,451 [6]. However, in our study brain ChE inhibition leveled off at about 800 LD50/ft^2 (1600 granules applied/ft^2) (Fig. 1).

Birds may reach an asymptote in their consumption of pesticide granules for several reasons. At low application rates, the probability of ingesting a granule may increase as granule availability increases because of an increase in the frequency of encountering granules, or an increase in granule visibility. Beyond a certain point, however, an increase in granule availability no longer affects the probability of birds picking up granules. Birds might also reach a satiation level with granules that are ingested as grit or food, which would also result in an asymptotic response.

An understanding of the relationship between avian risk and granule availability is not only important for accurately assessing the risk that granular pesticides pose to birds but also for developing granular pesticide formulations that might reduce avian risk. For example, reducing the pesticide load per granule may increase the number of granules per square foot, but it also could reduce the risk that the granules pose to birds because a bird might not ingest enough granules to cause an adverse effect before reaching satiation [8].

The relationship between avian risk and granule availability should be investigated for other granular carriers because recent research has demonstrated that the physical characteristics of different carrier types can affect avian risk from granular pesticides. For example, the integrity of different granules in house sparrow gizzards widely vary, which may influence granule consumption rates [18]. Further, when house sparrows were experimentally exposed to fensulfothion on silica, clay, or corncob granules, the amount of mortality and plasma and brain cholinesterase inhibition depended, in part, on the granule type [8]. Thus, it seems possible that the different granule characteristics also may affect the relationship between avian risk and granule availability.

Species differences also should be investigated. Because bird species differ in their grit consumption rates [17], satiation levels also would probably differ. In addition, bird species differ in their sensitivity to a given compound [13]; thus, some species might not experience adverse effects before reaching a satiation level whereas others could.

Additional pen studies that investigate carrier type and species differences, followed by field evaluations, could further elucidate the relationship between avian risk and granule availability. Such studies would provide information that could generate a risk exposure model that would more accurately predict avian risk from granular pesticides.

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Table 1. The mean (± 1 standard error) of brain cholinesterase activity and of the number of birds dying and exhibiting each behavioral symptom for each application rate.

	Application Rate (LD50/ft^2)								
	0	50	100	200	400	800	1200	1600	2000
Brain ChE Activity (μmol/min/g tissue)	33.8 (1.6)	27.3 (1.7)	23.0 (1.6)	22.6 (3.3)	20.3 (1.8)	19.2 (1.1)	16.2 (1.7)	18.7 (1.2)	17.8 (2.7)
Mortality	0.0 (0.0)	1.0 (0.0)	2.0 (1.0)	2.0 (1.0)	2.0 (1.5)	3.0 (1.0)	2.5 (0.5)	2.5 (0.5)	1.0 (1.0)
Behavior									
Ataxia	0.0 (0.0)	2.0 (2.0)	0.5 (0.5)	2.5 (1.5)	2.0 (1.0)	2.0 (1.0)	2.0 (1.0)	2.5 (0.5)	2.5 (1.5)
Catatonia	0.0 (0.0)	1.0 (1.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)	1.0 (1.0)	0.5 (0.5)	0.0 (0.0)	0.0 (0.0)
Hyporeactivity	0.0 (0.0)	0.5 (0.5)	1.0 (1.0)	1.0 (1.0)	1.5 (1.5)	0.0 (0.0)	1.5 (0.5)	1.5 (1.5)	1.5 (1.5)

Figure Captions:

Figure 1. Relation between the natural logarithm of application rate and mean brain cholinesterase activity. Capped vertical bars denote 1 standard error.

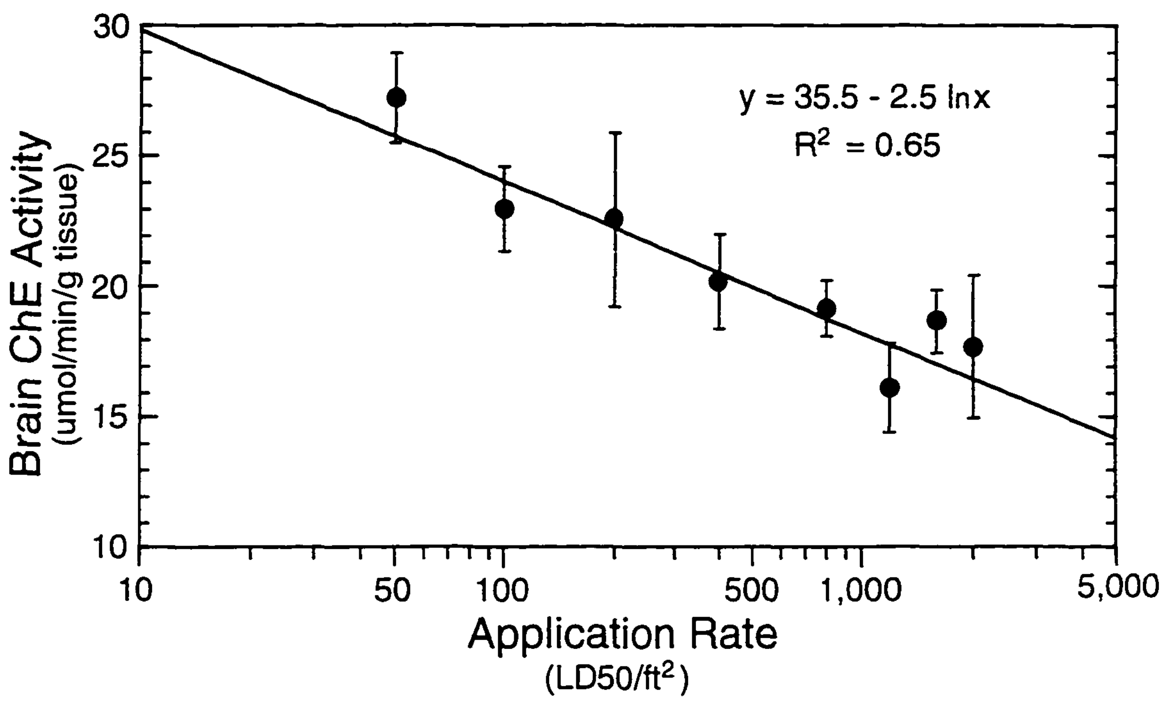
Brain ChE Activity
(umol/min/g tissue)
30
25
20
15
10
10
50
100
500
1,000
5,000
Application Rate
(LD50/ft2)
y = 35.5 - 2.5 lnx
R2 = 0.65

Figure 1

CHAPTER 5: BIRD RESPONSE TO GRIT/GRANULE CHARACTERISTICS AND ITS IMPLICATIONS FOR AVIAN RISK REDUCTION

A paper to be submitted to Environmental Toxicology and Chemistry

Tamara R. Stafford and Louis B. Best

Abstract–Granular pesticides pose a hazard for birds because of the potential for birds to ingest the granules. Avian risk from granular pesticides is currently assessed by using a risk index that is based on both the acute toxicity of the pesticide and the number of granules available per unit area on the soil surface after application ($LD50/ft^2$). Recent evaluations demonstrate that avian response to pesticide granules as a source of grit also can influence avian risk from pesticide granules. Herein we review the limitations of the current risk index, summarize the extant information regarding bird response to grit/granule characteristics (i.e., granule carrier type, color, size, shape, and surface texture, and pesticide load per granule), and suggest additional factors that could be included in a risk exposure model and how they might be represented in such a model. Sufficient information exists to begin considering inclusion of granule carrier type, color, and size, and pesticide load per granule in risk estimates. Refining the risk exposure model should be an ongoing process and not deferred until other factors that may influence avian risk are assessed. Additional evaluations regarding avian ingestion of pesticide granules as grit, as well as risk to birds from granular pesticides through other routes of exposure, are needed to refine the risk assessment process.

INTRODUCTION

The extensive use of granular pesticides throughout the United States poses a particular hazard for free-ranging birds because of the potential for them to ingest the granules. Both the high toxicity of granular pesticides [1,2] and the availability of the granules on the soil surface after application [3,4] has prompted concern on the part of the Environmental Protection Agency (EPA). In general, the EPA characterizes ecological risk by using the quotient method, which considers both toxicological data and exposure data (Risk quotient = Exposure / Toxicity). This concept has been used to generate an index specific for assessing acute avian risk from granular pesticides by using mortality as the endpoint. The risk index is based on both the acute toxicity of the pesticide and the number of granules available on the soil surface after application and is expressed as $LD50/ft^2$ [5]. The EPA's level of concern (LOC) for acute risk to birds from granular pesticides is 0.5 $LD50/ft^2$ (0.1 $LD50/ft^2$ for endangered species) [6], and risk to birds from a given granular pesticide is assumed if the quotient exceeds the LOC [7]. There are, however, factors other than acute toxicity and granule availability that contribute to avian risk (see below) that are not included in the risk index. Thus, the index may be misleading and is undoubtedly incomplete when predicting the risk to birds from granular pesticides.

The EPA recognizes that there are limitations with the risk index and identified several categories of information that could aid in refining the current approach, one of which was to evaluate the mechanisms of granular ingestion [5]. Best and Fischer [8] summarized the potential routes of granular ingestion and reported that there were limited data regarding grit use by birds and the degree to which pesticide granules might be perceived as grit by birds. Subsequent to Best and Fischer's review, considerable work has been done regarding avian grit-use patterns and bird response to grit and granule characteristics. The information from this research could be useful in refining the current risk index, and it also could aid industry in developing granular pesticide formulations that are less hazardous to birds. It is, therefore, important to synthesize the research that pertains to avian consumption of granules as grit and to evaluate its relevance to risk mitigation and refinement of the risk index. The purpose of our paper is to (a) briefly review the limitations of the current risk index and illustrate the importance of timely refinements, (b) summarize the extant information regarding bird response to grit/granule characteristics, (c) provide insights for reducing risk through the development of granular formulations that are less hazardous to birds, (d) identify future research needs to further our understanding of bird response to granule characteristics, and (e) make suggestions for additional factors that could be included in a risk exposure model and how they might be represented in such a model. Thus, the revised model would include a third element: factors that influence the likelihood that birds will consume pesticide granules as grit once they are encountered.

LIMITATIONS OF THE CURRENT RISK INDEX

Acute avian toxicity and granule availability

There are uncertainties associated with using each of the two factors contained in the current risk index for assessing the potential hazard to birds from granular pesticides. For example, acute toxicity data are generated in the laboratory from LD50 tests that use adult mallards (*Anas platyrhynchos*) and northern bobwhite (*Colinus virginianus*) as test species. These two species may be poor indicators of acute avian intoxication because they are not representative of the toxicological sensitivities of most North American bird species [9,10], nor are they representative of the different ages of birds. In addition, LD50 data usually are obtained by using the technical material rather than the formulated product, and the two can differ in toxicity [2]. It is, therefore, imprecise to extrapolate results from laboratory studies to field conditions.

Accurately estimating the number of granules available to birds per unit area after application also can be difficult. There are several different application methods for granular pesticides, which are accompanied by various methods to incorporate the granules into the soil at application (see [8] for a discussion). Despite efforts to incorporate the granules, granules are exposed on the soil surface to some extent after application [3,4] and the efficiency of incorporating the granules varies for each application/incorporation method. Factors considered in the estimate of the number of exposed granules include the application rate, the application method, % active ingredient of the formulation, and incorporation efficiency. EPA acknowledges that the number of granules on the soil surface can vary depending on carrier type, application rate, and method, etc. (O'Brien, 1987 in [5]) but they do not have the data needed to determine the range of variation for these factors [5].

Another limitation regarding estimation of granule availability is that the current index only considers granule availability immediately after application and does not account for changes in availability over time. Rapid incorporation of pesticide granules has been documented in pen studies with simulated soil surfaces and was attributed to both bird foraging activities and simulated rainfall [11] (Stafford and Best, unpubl. data). Further, counts of exposed silica granules in Iowa cornfields 11 days after application had declined to 1 to 12% of the day 0 count, and the decrease was attributed mainly to incorporation of the granules into the soil by rainfall [12]. Granules also can be displaced by wind [13] and/or covered by wind-blown material.

Assumptions used in the current risk index

There are several assumptions and conditions used by the EPA in evaluating acute avian risk from granular pesticides [5,7] and there is some concern regarding their accuracy. For example, it is assumed that avian risk will increase as granule availability increases. Exposing captive house sparrows (*Passer domesticus*) to progressively greater application rates (increase in the number of granules/ft^2), however, did not result in a linear increase in adverse effects; rather, brain ChE inhibition leveled off, indicating that the relationship between avian risk and granule availability can be asymptotic (Stafford and Best, unpubl. data). In addition, increases in the application rates and availability of silica granules applied to cornfields did not seem to increase consumption rates by birds [12]. Thus, considering a granular pesticide that has a greater number of granules/ft^2 after application to be more hazardous than one that has fewer granules/ft^2 after application may not be accurate if the LD50/ft^2 for both pesticide formulations exceed the granule consumption level of the birds. The hazard posed by a granular pesticide may be more a function of the concentration of toxicant per granule (i.e., pesticide load per granule) rather than the amount of toxicant available per unit area [12].

The EPA also assumes "that the amount of toxicant available to birds per unit area provides an indication of the actual amount of granular pesticide that birds could ingest" (p. 7) [5]. The assumption that the birds could ingest all of the granules within one square foot presupposes that (1) birds are foraging only in the treated area (i.e., pesticide bands), and (2) birds are not ingesting particles other than pesticide granules. And by using LD50 in the estimation of risk, it is further assumed that birds are ingesting the granules within one square foot as an acute dose (all at once). There are potential problems with these assumptions. For example, because the EPA considers the treated area to be "the most disturbed area in the field and the focus of bird foraging activity" (p. 37) [5], the area between pesticide bands is not figured into the calculation of toxicant per unit area. It is unlikely, however, that birds forage exclusively within the treated area and ingest only pesticide granules. Thus, using only the treated area in calculating a risk quotient might exaggerate the actual level of risk (unpubl. correspondence from the American Crop Protection Association to the EPA, Nov. 30, 1992). In addition, the estimated number of exposed granules/ft^2 following application of 14 granular pesticide formulations ranged from 295 to 14,451 [5], and it is doubtful that birds would, or could, ingest such large numbers of granules, especially as an acute dose.

Limitations in risk mitigation

Currently, risk reduction measures are only evaluated within the confines of the risk index thus limiting the measures that are acceptable to mitigate risk. At present, only two strategies can be pursued to reduce avian risk that are accounted for in the model: (1) developing less toxic pesticides or (2) reducing granule availability by either decreasing the application rate or increasing incorporation efficiency. Incorporation efficiency has been studied to some extent and several methods have been developed that might improve granule incorporation. For example, prototypes of several incorporation devices were made and tested in the field, some of which reportedly resulted in placement of 93 to 100% of the granules beneath the soil surface [14]. Although these measures may serve to reduce risk, there may be other opportunities to reduce risk, such as altering granular appearance (see below) that are not accounted for in the risk assessment process using the current risk index.

Other criteria influencing risk not included in the model

The current risk index does not include many other factors that could influence avian risk, such as bird behavior, weather conditions, and potential routes of exposure to pesticide granules. Several aspects of bird behavior could influence the possibility that a bird might be exposed to a granular pesticide, such as feeding ecology (foraging location, food items selected, etc.), breeding ecology (nest location, dietary changes during breeding season, etc.), and habitat use (habitat preference, migratory vs territorial resident, etc.) [5,8].

Weather conditions can influence granule availability, either by granule incorporation, displacement, or disintegration. Weather may further influence the potential for avian exposure in several ways. Reports of repeated poisonings of birds after foraging in treated fields that were flooded or that developed puddles after pesticide application [15] indicate that birds could be exposed to the pesticide either dermally or via drinking water. In addition, avian foraging activities may increase under cool, wet weather conditions [16,17], thus increasing grit/food consumption rates and the likelihood of ingesting granules. Weather also may decrease food availability (e.g., drought conditions could limit primary food sources), which might increase granule ingestion as food as birds explore alternative food sources (Stafford and Best, unpubl. data). Further, pesticide granules made available to captive house sparrows on wet soil posed less risk to the birds than on dry soil (Stafford and Best, unpubl. data) (see below), indicating that the risk from pesticide granules can be altered under different moisture conditions. Alterations might occur due to a change in either the appearance of the granules that decreases the likelihood of ingestion or in the toxicity of the granules (i.e., the pesticide is washed off the granule).

In addition to bird behavior and weather conditions, risk can be influenced by the route(s) through which birds are exposed to granular pesticides. Potential routes of exposure include inhalation, dermal absorption, and granule ingestion (see [8] for a detailed discussion), and it is possible that several routes of exposure might operate simultaneously. In addition, the routes of exposure might shift (see below), depending on the season, weather, food availability, etc. Some exposure routes may be more important than others in affecting avian risk, and it is important to determine which routes are more likely to occur. Exposure via inhalation is probably unlikely because granular pesticides were developed to address human safety concerns and are, therefore, formulated to produce as little dust as possible when being handled and applied. The potential for dermal absorption of pesticide from granular formulations under field conditions is largely unknown, but the dermal toxicity of granular pesticides tends to be lower than that for liquid formulations [18].

Reports of the recovery of both pesticide granules and pesticide residues in the gastrointestinal tracts of birds [19,20,21] indicate that granules are ingested by birds, and ingestion is considered the most potentially hazardous route of exposure [22]. Pesticide granules could inadvertently be ingested by birds in several ways, such as by adhering to selected food items or by being picked up with extraneous material during feeding. These are probably chance events. Birds also may ingest animal prey or plant items containing pesticide residues, but this may not be a primary route of exposure. For example, plant material might be contaminated by granular pesticides (via translocation to leaves, shoots, etc.), but due to the amount of material that a bird would have to ingest to have an adverse effect, this is probably not a significant source of risk [8].

The intentional ingestion of pesticide granules by birds is probably of greater concern for avian risk, which might occur if the birds either mistake pesticide granules for food or consume them as grit. A comparison between various granule carrier types used for corn rootworm (*Diabrotica* spp.) control and the seeds commonly found in midwestern rowcrop fields showed that seeds were larger and of different shapes than the granules (Best, unpubl. data). This suggests that many carrier types may not be confused with the seeds normally consumed by birds. But other carriers may be similar to food. For example, corncob granules resemble corn fragments, a food consumed naturally by some bird species [23]. Also, some granule types are similar to particles naturally consumed by birds as grit (see below). Intentional consumption of pesticide granules by birds, either as food or grit, is probably a likely route of exposure.

Although we recognize that there are many factors that can influence risk from granular pesticides, much of the work that has been done in recent years has concentrated on avian response to grit and the likelihood that birds might perceive pesticide granules as a source of grit. Consequently, we will focus our discussion on the ingestion of granules as grit as the primary route of exposure and on the granule characteristics that affect that route.

Value of the current index in the risk assessment process

It is clear that the data base for the existing risk index is far from complete. Although incomplete, it is practical (i.e., cost effective) to use data that are available to obtain rough estimations of risk via a mathematical model. It is important, however, to continue to fill the information voids inherent in the risk index to improve its accuracy in assessing avian risk from granular pesticides. Further, new information should be incorporated into the index as it becomes available instead of waiting for all of the information voids to be filled. Thus, refinement of the risk index should be an ongoing process.

BIRD RESPONSE TO GRIT/GRANULE CHARACTERISTICS

Natural grit-use patterns

The likelihood that birds may ingest pesticide granules as a source of grit probably depends on the similarity of a granule carrier type to grit. It is therefore important to understand the grit characteristics that influence natural grit use by birds, and the influence that diet, body size, and season have on grit consumption. Investigations of grit use by free-ranging birds have shown several patterns regarding the frequency and amounts of grit consumed, the types of particles selected (size, shape, surface texture, composition, and color), and grit retention and turnover in the gizzard (see [24] for a discussion).

The frequency and amount of grit used by birds varies greatly both within and among species [25,26] and can be influenced by age. Nestlings of many species are fed grit by their parents (e.g., [27,28]) and the amount of grit used by juveniles and adults may be more [29,30] or less [31,32] than that of nestlings, depending on the species. The quantity of grit-use also can be influenced by avian diet because grit acts as a grinding agent [33] and can provide mineral supplementation [34,35]. For example, greater grit use is evident when diets consist of hard foods, such as seeds and other coarse plant material, or hard-bodied insects [26,31,35,36]. Further, the amount of grit used by birds is influenced by grit size and grit retention in the gizzard (see below).

Size seems to affect the likelihood that a particle will be selected by birds as grit. Many avian species have shown preferences for certain ranges of grit size, which can differ greatly among species, both in the average grit size and the width of the range [25, 36]. Several factors seem to contribute to grit-size preference, including bird body weight, diet, and season. Generally, a larger bird will consume larger grit [26]. Also, the consumption of harder foods results in the selection of larger grit. For instance, spanish sparrows (*Passer hispaniolensis*) select larger grit during the summer when their diet consists mainly of cereal seeds and insects compared with the winter months when their diet consists of softer seeds [31]. In addition, greater amounts of large grit were ingested by white-tailed ptarmigans (*Lagopus leucurus*) during seasons when hard to digest foods were eaten [37]. Thus, grit-size preference also can be affected by the season. Further, an inverse relationship sometimes exists between mean grit size and the number of grit particles in the gizzard, indicating that birds selecting smaller grit use more particles [25,31,35,36] (Best, unpubl. data).

Grit shape and surface texture also may influence grit selection. The shapes and surface textures of particles found in the gizzards of free-ranging birds varied from spherical to oblong and from angular with sharp points to well-rounded with some species being more specialized toward certain shapes and surface textures than others [25]. Most of the particles found in the gizzards, however, was intermediate between having sharp, irregular surfaces and having smooth rounded surfaces. Gizzards of free-ranging house sparrows were reported to contain more angular grit particles when their diet consisted predominantly of insects compared with a diet consisting primarily of seeds [36], indicating that diet may influence the selection of grit with specific shapes and surface textures.

Although little information is available regarding the effects of grit composition and color on grit use, these characteristics seem to influence particle selection. Grit found in the gizzards of free-ranging birds consisted mainly of quartz particles (e.g., [34,37]), which may indicate a preference for quartz. On the other hand, quartz is often readily available, and it may tend to stay in the gizzard longer than other particles because of its hardness and insolubility [24]. Feldspar has also been found in birds' gizzards (e.g., 37,38]) and is also hard and insoluble, but it is not found as frequently as quartz. Further, birds consume items other than stone or rock fragments which seem to function as grit, including hard seeds, hard insect parts, shell fragments, bones, etc. (e.g., [34,39,40,41]). Gizzards also have been shown to contain particles of various colors ranging from clear (colorless) to black, and earth-tone hues (grays and browns) predominated [42]. The color of grit selected by birds may depend on both the availability and the conspicuousness of the particles (e.g., [43,44,45]).

Birds can retain grit in their gizzards for extended periods (e.g., [46,47]), and although the mechanisms are largely unknown, several factors seem to influence retention time, including grit availability, size, and diet. When grit is readily available, birds ingest and eliminate considerable amounts of grit daily [31,36,37], whereas birds without ready access to grit tend to reduce grit elimination (e.g., [46]). Other studies have indicated that when larger grit is consumed, fewer particles are ingested and retained [21,35,36]. Diet also may influence retention time. For example, hard food diets might increase the grit consumption rate, thus increasing the elimination rate [48]. In addition, retention time might be decreased with hard diets because they may accelerate grit particle disintegration and elimination [35].

Comparison of granule carriers and grit

A comparison of the granules of five pesticides (Furadan, Lorsban, Thimet, Counter, Dyfonate) demonstrated that the characteristics of granule carriers and grit can be similar [49]. For example, all five granule carriers were similar in size, and the mean granule sizes were at the lower extreme of the grit-size distributions found in the gizzards of cornfield birds. The shape and surface texture of Lorsban and Thimet granules were intermediate between spherical to oblong and angular with sharp points to well-rounded, as are many grit particles found in the gizzards of free-ranging birds. Further, Furadan granules were composed of silica thus demonstrating that some granules can be similar in composition to grit. In addition, the granules of Lorsban, Thimet, Counter, and Dyfonate had earth-tone hues, which shows a similarity between the color of some granules and grit. Other granule types (e.g., control release, complex cellulose) also have been used as pesticide carriers but their similarity to natural grit has not been evaluated. Because birds have preferences for certain types of grit (i.e., grit selection is not random), the potential to ingest pesticide granules as a source of grit probably depends, in part, on the overlap between the characteristics of natural grit and pesticide granules [8].

Experimental evidence of avian response to granule carriers

Because birds can potentially ingest pesticide granules as grit, it is important to determine which granule characteristics might influence granule ingestion. Information regarding the effect of granule carrier type, color, size, shape, surface texture, and pesticide load per granule on avian risk from granular pesticides has recently been evaluated.

Granule carrier type

The characteristics of different granule carrier types can vary (see above), which may influence the likelihood that a bird will select a granule as grit. Granule carrier types have been evaluated in terms of bird preferences, integrity in the gizzard once ingested, and how they are affected by weather conditions.

Several studies have demonstrated that birds prefer certain granule types when given a choice. Best and Gionfriddo [16] found that house sparrows preferentially consumed silica granules over five other granule types (heat-treated clay, bentonite clay, gypsum, corncob, and cellulose complex), and clay granules, although not compared directly to the other granule types, were selected more often than corncob, gypsum, and cellulose complex granules. After examining the color, size, shape, and surface texture of the pesticide granules, the birds' preferences for the different granules seemed to be related to the similarity between granule type and natural grit used by free-ranging house sparrows. Another experiment evaluated house sparrow preferences for silica, corncob, and heat-treated montmorillonite clay granules formulated with fensulfothion [11]. Greater adverse effects were seen in birds exposed to silica or clay granules compared with birds exposed to corncob granules, thus supporting the preference pattern observed by Best and Gionfriddo [16].

It was surprising in both experiments that birds did not show a greater preference for corncob granules because they resemble fragments of corn, a food used by many species of wildlife, including house sparrows [23]. Stafford et al. [11] suggested that the granule type preferences may have been confounded by the amount of feed offered to the birds (food was scattered *ad libitum* on the soil surfaces of the aviaries where the birds were confined). An additional study (Stafford and Best, unpubl. data) was thus conducted wherein the birds were given sufficient amounts of food to maintain their body weight, but the amount was decreased so that the feed was not as readily visible on the soil surface. In this subsequent study, greater adverse effects were seen in birds exposed to corncob granules compared with birds exposed to silica or clay granules. The change in the pattern of granule preference indicates that bird preferences for granular types are probably not constant and may vary under different environmental conditions. The change in preference from silica to corncob granules, for example, may demonstrate a shift in the route of exposure (i.e., consuming granules as food rather than grit), which might occur during times when food is less available for free-ranging birds.

According to the experimental data, it is evident that granule carrier type is a factor that can affect avian risk from granular pesticides, and the attractiveness to birds of granule types should be considered in the formulation design. Selecting a granule type that is less attractive to birds as a source of grit, such as cellulose-complex granules, would probably decrease the likelihood that a bird would ingest the granules as grit. Granule preference evaluations thus far, however, have been limited to house sparrows and bobwhites, and should be expanded to include other species that represent other feeding guilds and taxa. Also, preference tests have been limited to only a few granule types, and untested granules should be evaluated in terms of their attractiveness to birds prior to use. Avian preferences for a granule type could be evaluated by conducting pen studies similar to those previously performed. Granule type preferences also might be evaluated by comparing the characteristics of the granules (size, shape, color, etc.) to those of natural grit. The degree to which the granules resemble natural grit particles may indicate the potential for a bird to select the granule as grit.

The integrity of a granule carrier type might affect both granule consumption rates and granule availability. Best and Gionfriddo [36,50] found that the integrity of different granule types in house sparrow gizzards differed greatly. The granule types evaluated were silica, corncob, a heat-treated montmorillonite clay, a bentonite form of montmorillonite clay, gypsum, and a cellulose-complex granule, which represent a sample of the different granules that have been used to formulate granular pesticides. Silica granules persisted in the gizzards the longest and, on average, did not break down over the three day experiment. Heat treated clay and corncob granules were retained for several hours and the clay granules underwent considerable fragmentation. Bentonite, cellulose-complex, and gypsum granules disintegrated rapidly. These three would not be able to function as grit in the gizzard and probably would not provide a tactile stimulus in the gizzard as would grit. If granules that break down rapidly were consumed by a bird as a source of grit, they probably would not satisfy the bird's requirement for grit, and the bird might continue to consume additional granules (assuming that the pesticide did not alter the bird's appetite for grit). Thus, less integrous granules may increase avian risk from granular pesticides [8]. On the other hand, less integrous granules in the field might break down quickly when wet, and granules would be available to birds for consumption for a shorter period of time.

The integrity of a granule carrier type should be considered when formulating a granular pesticide. Granule integrity not only has the potential to influence granule availability and granule consumption rates, but it is also likely to influence the kinetics of toxicant release from the granule, which could influence both the length of time that the toxicant remains on the granule after application and the subsequent absorption of the toxicant if ingested [8]. There are several possible scenarios of granule breakdown and toxicant release that could apply either to granules in the field or in a gizzard: quick breakdown and quick release, slow breakdown and slow release, and slow breakdown and quick release. Granules that release the pesticide quickly would be less toxic to birds if ingestion occurred after the toxicant was released from the granule. On the other hand, granules might be more toxic to birds if they are ingested before the toxicant is released because the pesticide probably would be released quickly once in the gizzard, thus, the birds would receive the entire "dose" of pesticide in a short time or all at once. Each breakdown-release scenario should be evaluated to determine if one poses less risk to birds than another. Further, because the rate of release can potentially affect the absorption rate of the chemical, it is possible that the acute toxicity (LD50) of a chemical may vary depending on which granule type is used as the carrier. Thus, acute dosing studies for granular formulations might yield important information regarding the rate of release.

Not only do granule type preferences and integrity influence avian risk from granular pesticides, but weather conditions may interact with granule carrier types to influence risk. Stafford and Best (unpubl. data) offered captive house sparrows three granule types (silica, corncob, and heat-treated montmorillonite clay), formulated with fensulfothion, in bands on dry or wet soil surfaces. Pesticide granules on the wet soil were exposed to simulated rainfall after application. The risk from all three granule types was reduced under wet conditions but not to the same extent. The risk from corncob and silica granules was significantly reduced when presented to birds on wet soil whereas the risk from clay granules on wet soil was not. The simulated rainfall may have facilitated the release of the pesticide from the granules and, because granule types vary in composition, the pesticide may have been released from the different granule types at different rates (Stafford and Best, unpubl. data).

Weather also may influence avian risk by affecting granule consumption rates. Grit consumption rates seem to be greater under cool, wet conditions [16,17]; thus, more pesticide granules may be ingested when temperatures are cooler and/or there are greater amounts of precipitation. Weather effects on grit consumption rates, however, have thus far only been observed in captive birds and have not been reported for free-ranging birds.

Granule preferences and integrity are important to consider when selecting a granule carrier type, but finding a carrier that would nullify avian risk in all situations is unlikely for several reasons. (1) The risk posed by some carriers may change depending on the weather conditions. The "best" granule type when considering weather effects might be one that varies little in potential risk from one environmental condition to another, such as the heat-treated montmorillonite clay granule used by Stafford and Best (unpubl. data). (2) Another problem in selecting less hazardous carriers would be the difficulty in finding one that is unattractive to all species and to all individuals within a species. (3) The chemical properties of some pesticides can impose certain limits on the kinds of granules that can be used [8], and the ideal choice of carrier may not be usable for a given chemical. (4) Birds can be exposed to granular pesticides through routes other than ingesting granules as grit (see above). It is possible that more than one route of exposure may be operating simultaneously, or that routes may shift under different environmental conditions. Although finding an ideal carrier that poses no risk to birds may not be feasible, the use of available data regarding granule preferences and integrity when selecting a granule type may substantially reduce avian risk.

Because granule carrier types also may be mistaken for food, an evaluation of granules as a source of food might further help in choosing less hazardous granule types, e.g., comparing the characteristics of granule carriers and those of seeds commonly used as food by free-ranging birds. Efforts should focus on the guilds and taxa of avian species that would be most likely to be exposed to granular pesticides.

Color

Color vision is highly developed in birds, and birds have exhibited preferences for specific colors (e.g., [51,52,53,54]). The desire to deter birds from consuming grain crops, pesticide treated seeds, or rodent baits (e.g. [55,56,57,58]) has prompted much of the research concerning avian color preferences and, therefore, most research has focused on color preferences for food. Often, any unnaturally-colored food can act as a deterrent to consumption [57,59]. Much of the research on color preferences, however, indicate that black, blue, and(or) green food items are often avoided by birds (e.g., [53,56,60]).

Recent work indicates that birds also have color preferences for grit particles [42]. Captive house sparrows and bobwhite quail were offered glass particles (similar in composition to quartz and in the preferred grit size range for both species) of several colors (blue, green, yellow, red, brown, black, white, clear). Both species appeared to prefer yellow, green, white, and sometimes brown particles, whereas blue and black particles were selected the least, supporting the findings of the food-color preference research. Little work, however, has been done regarding avian preferences for colored pesticide granules. Best and Gionfriddo [16] found that a black graphite coating on gypsum granules may have deterred granule consumption by captive house sparrows. Further testing of gypsum granules [17] demonstrated that uncolored gypsum granules were preferred over black pigmented and graphite coated granules. In addition, Stafford et al. [11] offered house sparrows blue and white silica granules formulated with fensulfothion, but no clear color preference was evident.

Because birds have demonstrated both color preferences and aversions, coloring the granules so as to make them either more (if the goal is to induce consumption for aversive conditioning) or less attractive/visible (see [8] for a discussion) may decrease the likelihood of ingestion. Although color may be the easiest granular characteristic to alter, there are several factors that need to be considered before using color as a means to reduce avian risk from granular pesticides. First, not all species of birds consistently prefer and/or avoid the same colors. For example, domestic chicks (*Gallus gallus*) have shown preferences for orange and blue (e.g., [51,61]), whereas other avian species prefer blue the least (see above). In addition, a color that may be generally avoided by a species may be preferred by some individuals within the species [42]. Second, the visibility of various colors would differ against different soils, which might affect granule color preferences. However, background color may not be as important as the absolute color of the particle [42,59,62]. An evaluation of avian ingestion of colored granules against different backgrounds (i.e., different soils) is needed to understand the influence of background color on the attractiveness of different colors of granules to birds. Lastly, both the availability of dyes and pigments approved by the Food and Drug Administration and/or the high cost of certain dyes or pigments may hinder the use of a desired color. Despite the problems, coloring granules potentially could reduce avian risk from granular pesticides. Research regarding grit/particle color preferences should be expanded to include additional species and different environmental contexts (e.g., grit availability, soil color and texture, etc.) to determine how generalized observed color patterns are among avian species. In addition, field evaluations of colored granules could demonstrate the usefulness of altering granule color as a means to mitigate risk.

Size, shape, and surface texture

Free-ranging birds have demonstrated preferences for certain ranges of grit size, and grit and pesticide granule size overlap (see above); thus, there is a potential for birds to ingest granules as a source of grit on the basis of granule size. Little work is available, however, to demonstrate whether granule size significantly influences avian risk. Stafford et al. [11] offered house sparrows small (0.2 to 0.6 mm) and large (1.0 to 1.4 mm) silica granules formulated with fensulfothion. The two sizes represented the upper and lower size range of grit normally consumed by house sparrows [25] in an effort to determine whether birds preferred large or small granules. There were no clear indications that granule size influenced adverse effects from the pesticide. Because both granule sizes were in the range of natural grit size, however, it may be that neither size was preferred because they represented the extreme ends of the range. Adverse effects may have been more pronounced if the most preferred particle size had been made available to the birds.

Additional research may show that granule size is a factor to be considered when formulating granular pesticides, and that size might be adjusted to mitigate avian risk. There are size constraints, however, with granular pesticide formulations imposed by human safety factors (i.e., dust problems) and ease of handling and application (i.e., flow characteristics, etc.) [8]. Given the limitations for granule sizes, coupled with the wide range of grit sizes used by birds, it may be difficult to find "safer" granule sizes for pesticide formulations that would be infrequently used by birds [26]. Despite the problems, granule size distributions that have little overlap with grit size distributions might pose less risk to birds.

No research has been conducted to directly assess the influence of granular shape and surface texture on avian risk from granular pesticides. Best and Gionfriddo [63] evaluated the influence of shape and surface texture on grit selection by house sparrows and northern bobwhite and found that most birds preferred angular/oblong grit over rounded/spherical grit. In addition, Pank [59] found that when some bird species were offered colored seeds, seeds colored with agents that altered the surface texture of the seed were preferred the least. It is possible, therefore, that altering granule shape and surface texture could influence the likelihood that a bird would ingest granules as grit. Additional research regarding the influence of shape and surface texture is needed to determine the utility of altering granule shape/surface texture as a way to reduce avian risk. As with granule size, formulation constraints exist for granule shape and surface texture because they might affect flow characteristics. Plus, it may not be possible to alter the shape of some carriers (see [8] for a discussion).

<u>Pesticide load per granule</u>

The amount of pesticide (load) in/on each granule is another factor that can contribute to avian risk. For example, when captive house sparrows were offered different loads of fensulfothion (1/2, 1/8, and 1/32 the LD50 per granule) on three different granule carriers (silica, heat-treated montmorillonite clay, and corncob), greater adverse effects occurred when the birds were exposed to the higher pesticide load per granule [11] (Stafford et al., unpubl. data). It may be possible, therefore, to reduce avian risk from granular pesticides by reducing the pesticide load per granule. Although reducing the load per granule would result in an increase in the number of pesticide granules per square foot (assuming that the LD50s per square foot remain the same), it is possible that birds would not ingest enough granules to cause adverse effects for at least two reasons: (1) birds might only consume granules until their grit (or food) appetite is satisfied thus reaching a satiation level before ingesting enough granules to cause adverse effects, and (2) the effects of the pesticide already ingested might lessen their grit-seeking behavior (or lessen their appetite for food) before lethal concentrations were consumed.

The feasibility of reducing pesticide load per granule as a means to reduce avian risk would depend on the grit/granule consumption rate by birds, the toxicity of the pesticide, the rate at which birds detoxify sublethal doses of pesticide, and mechanical constraints (i.e., ability of conventional machinery to apply more granules per unit area) (see [8] for a discussion). Although reducing the pesticide load per granule in pen studies has significantly reduced adverse effects to birds, it is important to determine if reducing the pesticide load per granule will have the same outcome in the field.

REFINEMENT OF THE CURRENT RISK EXPOSURE MODEL

Because avian response to pesticide granule characteristics and pesticide load per granule are not accounted for when assessing risk to birds from granular pesticides, risk estimates derived from the current index are likely misleading. For example, the application rates used in several experiments [11] (Stafford and Best, unpubl. data) resulted in $LD50/ft^2$ of 50 to 2000, far above the EPA's level of concern (0.5 $LD50/ft^2$). Furthermore, some of the experimental treatments were considered to be "worst case scenarios" (e.g., high toxic loads per granule, use of silica granules that are highly attractive to birds). If the EPA assumption that the number of granules available on the soil surface is indicative of the number of granules that birds can ingest, then all birds in each experiment would have been expected to die. The average mortality in any experiment, however, never exceeded 20%. Because avian risk potentially can be inaccurately estimated, it is important to incorporate information regarding granule characteristics into either the current risk index or a new risk assessment model to obtain more realistic estimates of risk.

Representing granule characteristics in the risk index

Before including additional factors in the risk assessment process, there should be sufficient experimental evidence to show that the factors can influence avian risk from granular pesticides. Birds have shown preferential ingestion of different granule carrier types, objects of different colors, and particles in specific size ranges. In addition, varying the concentration of toxicant per granule can affect the hazard that granular pesticides pose to birds. Thus, granule carrier type, granule color, granule size, and pesticide load per granule should be considered for inclusion in the risk assessment process. There are additional factors that may significantly influence avian risk, such as granule integrity (in the GI tract or environment), shape, and surface texture, but sufficient experimental evidence is not yet available regarding their influence on avian risk to discuss their inclusion in a risk assessment model at this time.

Granule characteristics that can affect avian risk must be quantified in some manner before they can be used as terms in a risk assessment model. One possibility is to establish a standard for each characteristic against which granular formulations under investigation could be compared. Differences between the standard and the granular formulation could be measured and factored into a model, and each granule characteristic would then contribute to the estimate of risk for the granular formulation. Differences between the granular formulation and the standard could be expressed as a quotient by dividing the measurement of bird responses to the granular formulation by the measurement for the standard. For example, bird preferences for granule type could be assessed by dividing the amount consumed of the granular formulation by the amount consumed of the granule type standard (see below). The effects of granule characteristics on avian consumption of the granules also could be evaluated simultaneously if the standard encompassed more than one granule characteristic.

Standards for granule characteristic comparisons should be similar to grit used by free-ranging birds. Because information about granule characteristics is not considered in estimates of avian risk currently used by the EPA, the index assumes that granular formulations do not affect avian consumption of pesticide granules, and that birds would be as likely to consume pesticide granules as they would to consume natural grit. If standards are selected that have the same potential for consumption by birds as does natural grit, then comparisons that show no difference between the standard and the granular formulation would result in a quotient of 1. If a risk exposure model is multiplicative, then including a term with a quotient of 1 would not affect the model (i.e., the granule characteristic in question does not influence avian consumption of granules). A quotient of less than 1 would indicate that the granule characteristic could decrease the potential risk of avian consumption of the granular formulation, and the term could be included in the model in such as way that the overall risk estimate would be lowered. Conversely, inclusion of quotients greater than 1 in the model could increase the risk estimate for the granular formulation. Comparing each characteristic against its own standard to generate a value that could be used to estimate risk assumes that each characteristic affects granule consumption by birds independently of other characteristics. At present, an assumption of independence seems reasonable given the absence of evidence to the contrary.

A standard for granule-type preference comparisons could be sand particles (quartz) because quartz is the most commonly used grit material by birds (see above). Avian preferences for a given granule carrier type could be compared to the standard by measuring the amounts that birds consume of each and by dividing the amount consumed of the granule carrier by the amount consumed of the standard. Clearly, the pesticide could potentially interfere with measurements of granule-type preference. Thus, evaluations of carrier types as formulated should not include the pesticide. Also, granular formulations often include inert coatings for various reasons (e.g., to adhere the pesticide to the granule), and these might change the granule color, texture, or some other attribute, which might influence preference. Thus, such coatings should be included when evaluating granule type. In addition, granule size for both the standard and the granular carrier should be the same so that granule-type preference effects are not confounded.

The effects of changing the color of granules with pigments or of applying inert coatings to the granules during the formulation process that change the color of the granules could also be evaluated. One possibility would be to use a neutral color (an earth-tone dye within the range of typical grit colors) as a standard against which to compare the formulation being tested. In such evaluations both the standard color and the color being evaluated would be formulated on the same granule type so that granule type effects would be held constant. It is possible, however, that the color standard may vary in hue when formulated on different granule types. In addition, the chemical properties of the dye may not be compatible with the chemical or structural properties of certain granule types. An alternative approach to a standard would be to use the same granule type used in the granule formulation being evaluated, but excluding the pigments and/or the coatings that alter the granule color. Although the color of the standard would be different for every comparison (i.e. variation in the natural color of granule types), this approach would demonstrate whether or not the color of the granular formulation being evaluated would influence avian preferences for the colored granules.

For granule size evaluations, a standard granule size distribution could be derived from available information regarding grit size use by free-ranging birds [25,26]. The proportional overlap between the size distribution of the pesticide granules being evaluated and the grit sizes naturally used could then be determined. If the granule size distribution completely overlapped the standard size distribution, the proportional overlap would equal 1. As with quotients that equal 1, including a proportion of 1 as a multiplicative term would leave a risk model unaffected. Size distributions of formulated products that partially overlap with the standard would indicate that granule size could increase the potential of avian consumption of the granular formulation, and the proportion of overlap should be included in the model in such a way that the overall risk estimate would be increased.

Evaluations of the effects of pesticide load per granule would be different from evaluations of granule carrier type, color, and size because the pesticide load does not affect avian preferences for granules (unless there is an avoidance response). Rather, the load affects the likelihood that a bird will suffer adverse effects from the pesticide after ingesting the granules. In assessing the effect that pesticide load per granule could have on avian risk, both the pesticide load per granule (LD50/granule) and the daily grit consumption rate need to be considered because both contribute to the amount of toxicant a bird might ingest. The total LD50s that a bird potentially might ingest could be estimated by multiplying the LD50/granule by the daily grit consumption rate. For example, a bird species that consumes on average 10 grit particles per day and that encounters granules with a pesticide load of 0.05 LD50, could potentially ingest an LD50 of 0.5. A value equal to 1 would indicate that the daily consumption of granules equals the number of granules that constitutes an LD50 for a species, and if added to a model as a multiplicative term, the risk estimate would be unaffected. A value less than 1 would indicate that a bird would receive less than one LD50 and would, therefore, be at less risk from the pesticide.

To date, both grit counts and the frequency of occurrence (i.e., the presence or absence) of grit in avian gizzards have provided indirect measures of grit consumption for several species [12,25,26]. We recommend using the average grit count per gizzard to estimate the daily grit consumption rate. Grit counts would provide more sensitive estimates of the daily grit consumption rate than would frequencies of occurrence because birds could have similar grit occurrence in their gizzards (e.g., 100%) but differ substantially in the number of grit particles. Using this estimate of the daily grit consumption rate probably represents the maximum risk to birds because it does not consider that the granules would likely be ingested throughout the day rather than all at once, and thus there potentially could be recovery from the ingested pesticide that would be unaccounted for in the calculation. Also, birds may lose their appetite for grit after ingesting pesticide granules and would be less likely to consume the amount of grit normally ingested in a day.

Relative importance of granule characteristics in assessing risk

Although granule characteristics can influence the likelihood that birds will ingest pesticide granules, it is unlikely that they do so to the same extent. When assessing avian risk, therefore, each characteristic should be weighted according to its relative contribution to risk, with greater weight being given to those characteristics that have a greater influence. The relative influence of granule characteristics on avian ingestion of granules has not yet been addressed. Thus, further experimentation is needed to determine the degree to which risk is affected by each characteristic. Further research should include field evaluations to demonstrate that the influence of granule characteristics on avian risk is the same in the natural environment as observed in artificial test systems. In addition, the availability of avian response data for each granule characteristic differs (i.e., more consistent information is available regarding avian response to granule type than avian response to granule size). Therefore, granule characteristics also could be weighted according to the level of confidence in the measurements of avian response to a given characteristic, with greater weight given to those characteristics that have more confirmatory data. Weighting granule characteristics on the basis of information availability would be necessary until further experimentation resulted in an equal level of confidence in the measurement of avian response to each characteristic. Further, granule characteristics should be weighted collectively according to their total contribution to the overall evaluation of avian risk (i.e., does avian response to pesticide granules as a source of grit influence risk more or less than other factors, such as granule availability). Birds are not only exposed to granular pesticides by intentionally ingesting granules as grit, but there are other possible routes of exposure (see above) that may influence risk. Thus, the relative contribution of each exposure route to avian risk should be accounted for in the risk assessment process.

Weighting the granule characteristics will not only increase the accuracy of the risk estimate, but it will also aid in determining the relative merits of altering the characteristics in terms of cost. The benefit of altering a granule characteristic of low weight (i.e., one that reduces risk minimally) might not outweigh the cost to make such an alteration, whereas a characteristic of high weight and low cost to formulate would be a more effective means to reduce risk.

Species differences in avian response to granule characteristics

Differences in the response to granular characteristics among avian species also should be considered when including granule characteristics in the risk assessment process because species differences would have a bearing on the selection of the standards to be used in evaluating granule characteristics. Avian response to some characteristics may be more generalizable than others. For example, most bird species would likely ingest silica granules as grit because the grit found in avian gizzards is often similar to silica (i.e., quartz) (e.g., [34,37]). Experimental evidence also seems to suggest that some avian species may show a similar pattern for color preferences (e.g., [42,53,56,60]), but further evidence is needed to determine whether or not the same color preference pattern is common among birds that may be exposed to pesticide granules. Conversely, acute toxicities, grit size preferences and daily grit consumption rates typically are species specific [25,26,64].

If the response by birds to a given granule characteristic does not have a general pattern, it is important to determine how the available information could best be used in selecting both granule characteristic standards and test species. One possibility would be to use all available data to encompass the known range of avian response. For example, a standard granule size distribution could be produced from a composite of the grit-size ranges documented for birds thus far. The grit size ranges used also could be restricted to those bird species likely to be present in the treated area or those of the greatest management concern (i.e., endangered species). Another possibility would be to determine which species could serve as indicators to represent avian response to granule characteristics in general; the approach that has been used to address species differences in acute toxicity. The accuracy of risk estimates that include granule characteristic data greatly depends on the manner in which species differences are addressed.

Concluding comments

Because of the limitations inherent in the current risk index, the estimates of risk to birds from granular pesticides can be inaccurate. The EPA has acknowledged the limitations of the current approach and recognizes that the risk index could be improved if additional data were made available to fill some of the information voids [5]. Although the EPA recognizes the questionable accuracy of the current index in estimating avian risk, applications of granular pesticides that result in greater than 0.5 LD50/ft^2 are considered to pose a high risk to birds and can be subject to regulatory action [65]. Because decisions regarding the registration of granular formulations can be based on information generated by the risk index, it is important to improve the ability of the index to assess the risk of granular pesticides to birds. It is also important that improvements to the index be made as information becomes available rather than waiting until all of the limitations are addressed. Evaluations of avian response to pesticide granules as a source of grit, although incomplete, have provided information that can and should be utilized now to refine the risk index. Continued evaluations regarding avian ingestion of pesticide granules as grit, as well as exposure to the pesticide through other routes, are further required to refine the risk assessment process.

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CHAPTER 6: GENERAL CONCLUSIONS

Both granular carrier type and pesticide load per granule had an influence on the risk to house sparrows from the granular pesticide. Silica and clay granules posed a greater risk to the birds than did the corncob granules, and the risk from the granular pesticide decreased as the pesticide load per granule decreased. No conclusions could be made regarding the effects that granular size and color might have on avian risk. It was thought that offering food to the birds *ad libitum* may have interfered with the magnitude of the effects. In addition, the severe weather during the spring and summer of 1993 may have interfered with the results.

Corncob granules posed greater risk to house sparrows than did silica or clay granules when birds were given less food, suggesting that the risk from some granular carrier types may be greater for free-ranging birds in years when the natural food supply is less and birds must search for alternative food sources. In addition, soil moisture had a significant influence on avian risk from the granular pesticide, thus indicating that the severe weather during the previous summer could have influenced the results of the earlier experiments. Pesticide granules on wet soil posed less hazard to house sparrows than did granules on dry soil. Wet soil did not, however, reduce risk to the same extent for all three carrier types. The risk from corncob and silica granules was significantly reduced, whereas the risk from clay granules was not. Granular pesticide formulations should include carrier types that not only pose the least risk to birds but that also vary little in risk from one environmental condition to another.

House sparrows exposed to fensulfothion formulated on silica granules showed an asymptote in brain cholinesterase inhibition, demonstrating that the relationship between avian risk and granule availability is not necessarily linear. The assumption of linearity implicit in the risk exposure model currently used may result in the overestimation of risk for granular pesticides that have a high number of granules exposed per unit area after application.

The information now available regarding avian response to pesticide granules as a source of grit could be used for both risk mitigation and assessment. Risk mitigation measures could involve selecting granular carrier types that are less attractive to birds in terms of their composition, color, and size. In addition, the LD50/ft^2 risk index could be refined by quantifying certain characteristics of granules (granular carrier type, color, and size) and including them in the model, thus increasing the accuracy of predicting the risk that granular pesticides pose to birds. Refinement of the risk index should be an ongoing process as new information becomes available.

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